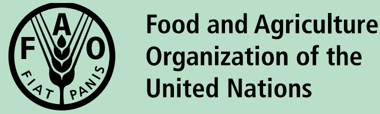
Pesticide residues in food — 2012

Joint FAO/WHO Meeting on Pesticide Residues

EVALUATIONS 2012

Part II — Toxicological



Pesticide residues in food — 2012

Toxicological evaluations

Sponsored jointly by FAO and WHO

Joint Meeting of the
FAO Panel of Experts on Pesticide Residues
in Food and the Environment
and the
WHO Core Assessment Group on Pesticide Residues

Rome, Italy, 11–20 September 2012

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* First full evaluation
** Evaluated within the periodic review programme of the Codex Committee on Pesticide Residues

2012 Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues

Rome, 11–20 September 2012

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Abbreviations used

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and elimination
ADP	adenosine diphosphate
ae	acid equivalent
AFC	antibody-forming cell
AHH	aryl hydrocarbon hydroxylase
ALP	alkaline phosphatase
ALT	alanine aminotransferase
APTT	activated partial thromboplastin time
ARfD	acute reference dose
AST	aspartate aminotransferase
ATP	adenosine triphosphate
AU	area unit
AUC	area under the plasma concentration–time curve
BMD	benchmark dose
BMD_L	lower-bound confidence limit on the benchmark dose
BMDS	benchmark dose software
BrdU	5-bromo-2′-deoxyuridine
BROD	benzyloxyresorufin *O*-dealkylase
BSP	bromosulfophthalein
BUN	blood urea nitrogen
bw	body weight
CAS	Chemical Abstracts Service
CCPA	4-chloro-2-carboxyphenoxyacetic acid
CHL	Chinese hamster lung
CHO	Chinese hamster ovary
CI	confidence interval
C_{max}	maximum concentration in plasma
CMC	carboxymethylcellulose
CP	cyclophosphamide
CPIA	2-(4-chlorophenyl)isovaleric acid
cpm	counts per minute
CYP	cytochrome P450
2,4-D	2,4-dichlorophenoxyacetic acid
DMA	dimethylamine
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DTH	delayed-type hypersensitivity
ECG	electrocardiography
ECOD	ethoxycoumarin *O*-deethylase
ED	embryonic day
ED_{30}	effective dose associated with a 30% decrease in motor activity
EH	epoxide hydrolase
2-EHE	2-ethylhexyl ester

EMS	ethylmethanesulfonate
EOS	eosinophils
Eq	equivalent
EROD	ethoxyresorufin *O*-dealkylase
EU	European Union
F	female
F_0	parental generation
F_1	first filial generation
F_2	second filial generation
FAO	Food and Agriculture Organization of the United Nations
FSH	follicle stimulating hormone
FU	fluorescence unit
GA	glufosinate-ammonium
GABA	gamma-aminobutyric acid
GD	gestation day
GGT	gamma-glutamyl transpeptidase
GHS	Globally Harmonized System of Classification and Labelling of Chemicals
GLP	good laboratory practice
GS	glutamine synthetase
GSD	geometric standard deviation
GSH	glutathione (reduced)
GSSG	oxidized glutathione; glutathione disulfide
H&E	haematoxylin and eosin
Hb	haemoglobin
HCT	haematocrit
HGB	haemoglobin
HMCPA	4-chloro-2-hydroxymethyl-phenoxyacetic acid
HOBI-GT	4-hydroxybiphenyl-glucuronosyl transferase
HPLC	high-performance liquid chromatography
IEDI	international estimated daily intake
IESTI	international estimate of short-term dietary intake
IgM	immunoglobulin M
IPCS	International Programme on Chemical Safety
ISO	International Organization for Standardization
ITC	intestinal tract contents
IU	international units
IUPAC	International Union of Pure and Applied Chemistry
iv	intravenous
JMAFF	Japanese Ministry of Agriculture, Forestry, and Fisheries
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
LAP	leucine aminopeptidase
LC-MS	liquid chromatography–mass spectrometry
LC_{50}	median lethal concentration
LD	lactation day
LD_{50}	median lethal dose
LDH	lactate dehydrogenase
LH	luteinizing hormone

LOAEL	lowest-observed-adverse-effect level
LOD	limit of detection
LOQ	limit of quantification
LSC	liquid scintillation counting
M	male
MCA	methylcholanthrene
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCPA	4-chloro-*o*-tolyloxyacetic acid
MCPB	4-(4-chloro-*o*-tolyloxy)butyric acid
MCV	mean corpuscular volume
MHB	2-hydroxy-4-methylphosphinico-butanoic acid
MMAD	mass median aerodynamic diameter
MMC	mitomycin-C
MOA	mode of action
MPA	2-methylphosphinico-acetic acid
MPB	4-methylphosphinico-butanoic acid
MPP	3-methylphosphinico-propionic acid
mRNA	messenger ribonucleic acid
MTD	maximum tolerated dose
MUF-GT	4-methylumbelliferone-glucuronosyl transferase
MW	molecular weight
NA	not applicable
NADPH	nicotinamide adenine dinucleotide phosphate (reduced)
NAG	*N*-acetyl-glufosinate
ND	not detectable
NEUTA	neutrophils
NK	natural killer
NMDA	*N*-methyl-D-aspartate
NMR	nuclear magnetic resonance
NOAEC	no-observed-adverse-effect concentration
NOAEL	no-observed-adverse-effect level
NOEC	no-observed-effect concentration
NOEL	no-observed-effect level
NS	no sample
NZW	New Zealand White
OECD	Organisation for Economic Co-operation and Development
OET	open epicutaneous test
OR	odds ratio
P	parental generation; probability
PB	phenobarbital
PBacid	3-phenoxybenzoic acid
PEG	polyethylene glycol
PFC	plaque-forming cell
PND	postnatal day
PPAR	peroxisome proliferator-activated receptor
PPB	Perl's Prussian Blue

ppm	parts per million
PROD	pentoxyresorufin *O*-dealkylase
PSP	phenolsulfonphthalein
PT	prothrombin time
PTT	partial thromboplastin time
PTU	propylthiouracil
QA	quality assurance
RBC	red blood cell
ROS	reactive oxygen species
RR	relative risk; rate ratio
RT	retention time
S9	9000 × *g* supernatant fraction of rat liver homogenate
SCE	sister chromatid exchange
SD	standard deviation; Sprague-Dawley
SI	stimulation index
SMA	spontaneous motor activity
SMR	standardized mortality ratio
SPE	solid-phase extraction
sRBC	sheep red blood cell
2,4,5-T	2,4,5-trichlorophenoxyacetic acid
$t_{½}$	half-life
T_3	triiodothyronine
T_4	thyroxine
T_4-UDP-GT	thyroxine uridine diphosphate-glucuronosyl transferase
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
TEPP	tetraethylpyrophosphate
TLC	thin-layer chromatography
T_{max}	time to reach maximum concentration in plasma
TMPA	2,2,3,3-tetramethylcyclopropanecarboxylic acid
TOCP	tri-*ortho*-cresyl phosphate
TOTP	tri-*ortho*-tolyl phosphate
TRR	total radioactive residue
TSH	thyroid stimulating hormone
TTC	threshold of toxicological concern
U	units
UDP	uridine diphosphate
UDPGT	uridine diphosphate glucuronosyl transferase
UDS	unscheduled DNA synthesis
USA	United States of America
USEPA	United States Environmental Protection Agency
UV	ultraviolet
V_d	volume of distribution
WBC	white blood cell
WHO	World Health Organization
w/v	weight per volume
w/w	weight per weight

Introduction

The toxicological monographs and monograph addenda contained in this volume were prepared by a WHO Core Assessment Group on Pesticide Residues that met with the FAO Panel of Experts on Pesticide Residues in Food and the Environment in a Joint Meeting on Pesticide Residues (JMPR) in Rome, Italy, on 11–20 September 2012.

Seven of the substances evaluated by the WHO Core Assessment Group (ametoctradin, chlorfenapyr, dinotefuran, fluxapyroxad, MCPA, picoxystrobin and sedaxane) were evaluated for the first time. Four compounds (bentazone, fenpropathrin, fenvalerate and glufosinate-ammonium) were re-evaluated within the periodic review programme of the Codex Committee on Pesticide Residues (CCPR). Reports and other documents resulting from previous Joint Meetings on Pesticide Residues are listed in Annex 1.

The report of the Joint Meeting has been published by the FAO as *FAO Plant Production and Protection Paper 215*. That report contains comments on the compounds considered, acceptable daily intakes established by the WHO Core Assessment Group and maximum residue limits established by the FAO Panel of Experts. Monographs on residues prepared by the FAO Panel of Experts are published as a companion volume, as *Evaluations 2012, Part I, Residues*, in the FAO Plant Production and Protection Paper series.

The toxicological monographs and monograph addenda contained in this volume are based on working papers that were prepared by WHO experts before the 2012 Joint Meeting. A special acknowledgement is made to those experts and to the Members of the Joint Meeting who reviewed early drafts of these working papers.

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned.

Any comments or new information on the biological properties or toxicity of the compounds included in this volume should be addressed to: Joint WHO Secretary of the Joint FAO/WHO Meeting on Pesticide Residues, Department of Food Safety and Zoonoses, World Health Organization, 20 Avenue Appia, 1211 Geneva, Switzerland.

TOXICOLOGICAL MONOGRAPHS
AND MONOGRAPH ADDENDA

AMETOCTRADIN

First draft prepared by
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Explanation

Ametoctradin (Figure 1) is the common name provisionally approved by the International Organization for Standardization (ISO) for 5-ethyl-6-octyl[1,2,4]triazolo[1,5-a]pyrimidin-7-amine, for which the Chemical Abstracts Service number is 865318-97-4. Ametoctradin is a fungicide that inhibits zoospore differentiation within the zoosporangium, the release of zoospores from the zoosporangium, the motility of any released zoospores and the germination of encysted zoospores. It acts by reducing the adenosine triphosphate content in these stages of development by binding to and inhibiting complex III of the respiratory chain in mitochondria of oomycetes.

Ametoctradin has not been evaluated previously by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) and was reviewed at the present Meeting at the request of the Codex Committee on Pesticide Residues.

Figure 1. Structure of ametoctradin

All studies evaluated in this monograph were performed by good laboratory practice–certified laboratories and complied with the relevant Organisation for Economic Co-operation and Development and/or United States Environmental Protection Agency test guidelines.

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

The absorption, distribution, metabolism and elimination of ^{14}C-labelled ametoctradin were investigated in male and female Wistar rats at doses of 20, 100, 500 and 1000 mg/kg body weight (bw) for plasma kinetics and 50 and 500 mg/kg bw for mass balance, tissue distribution and biliary excretion experiments. The experiments were performed with ametoctradin labelled in the pyrimidine and triazole rings. In order to achieve the required specific activity, the respective amounts of non-labelled material were added to the radiolabelled material. To facilitate elucidation of the structures of the metabolites formed, ^{13}C-labelled material was mixed with unlabelled ametoctradin at a ratio of 1:1 and added to the respective amounts of ^{14}C-labelled ametoctradin. The study design is summarized in Table 1.

The dermal absorption of ametoctradin in the formulation BAS 651 00 F was investigated in vitro in human and rat skin.

Blood/plasma levels study: ^{14}C-labelled ametoctradin was administered to groups of four male and four female Wistar rats in a single oral dose of 20, 100, 500 or 1000 mg/kg bw. Blood samples were collected at 1, 2, 4, 8, 24, 48, 72 and 96 hours after administration.

Following a single oral dose of ^{14}C-labelled ametoctradin, maximum plasma concentrations were observed 1 hour after dosing, except in females dosed with 500 mg/kg bw, for which maximum plasma concentrations were observed 2 hours post-dosing. Afterwards, plasma levels declined until sacrifice at 96 hours in all dose groups to values of about 0.01–0.2 µg equivalent (Eq) per gram plasma. Initial half-lives in plasma ranged from 2.13 to 2.91 hours for males and from 1.74 to 2.51 hours for females (Table 2). The area under the plasma concentration–time curve (AUC) values indicate a sex-independent internal exposure and show that there is not a linear correlation between the internal exposure (measured as total radioactivity) and increasing external dose.

Mass balance/excretion study: Groups of Wistar rats (four of each sex) received radiolabelled ametoctradin as a single dose of 50 mg/kg bw, a single dose of 500 mg/kg bw or a single dose of 500 mg/kg bw following by 14 repeated daily doses of unlabelled compound at 500 mg/kg bw. Urine was collected after 6, 12 and 24 hours, then at intervals of 24 hours up to 168 hours, and faeces were collected at intervals of 24 hours up to 168 hours. After 168 hours, animals were sacrificed, and tissues were collected.

Total recovery of radioactivity at 168 hours ranged from 91% to 110% of the administered dose. The main route of elimination was via faeces for all exposure regimens (from 73% to 102%; Table 3). Faecal elimination was nearly complete by 48 hours, with 71.9–82.1% for the low-dose group, 98.4–99.0% for the single high dose group and 82.4–94.2% for the repeated high dose group. Urinary excretion accounted for 4.8–21.8% and was also complete within 48 hours after administration. After 168 hours, only small amounts of radioactivity were present in tissues and carcass.

Table 1. Study design for biokinetic studies with ametoctradin

Test group	Dose (mg/kg bw)	No. of rats	Study design
Blood/plasma levels study			
Experiment 1	1000 (^{14}C)	4 M, 4 F	Retro-orbital blood sampling was conducted at 1, 2, 4, 8, 24, 48 and 72 h; exsanguinations were performed at 96 h.
Experiment 2	500 (^{14}C)	4 M, 4 F	
Experiment 3	100 (^{14}C)	4 M, 4 F	
Experiment 4	20 (^{14}C)	4 M, 4 F	
Mass balance/excretion study			
Experiment 5	500 (^{14}C)	4 M, 4 F	Excreta and expired air were determined by means of metabolism cages. Urine was collected after 6, 12 and 24 h and subsequently at 24 h time intervals. Exhaled air was collected for 48 h. Animals were sacrificed and tissues were collected at 168 h.
Experiment 6	50 (^{14}C and ^{13}C)	4 M, 4 F	
Experiment 7	500 (14× unlabelled followed by ^{14}C-labelled)	4 M, 4 F	
Tissue distribution study			
Experiment 8	500 (^{14}C)	12 M, 12 F	Sacrifice times, 500 mg/kg bw:
Experiment 9	50 (^{14}C)	12 M, 12 F	M: 1, 4, 16, 22 h
			F: 1, 2, 8, 20 h
			Sacrifice times, 50 mg/kg bw:
			M/F: 1, 2.5, 8, 20 h (3 of each sex per time point)
			Tissue analysis, total radioactivity
Biliary excretion study			
Experiment 10	500 (^{14}C and ^{13}C)	4 M, 4 F	Bile was collected at 3 h intervals for up to 72 h. In addition, urine and faeces were collected at 24 h time intervals up to 72 h. After sacrifice, stomach, gut and carcass were checked for remaining radioactivity. In addition, cage wash was checked for radioactivity.
Experiment 11	50 (^{14}C and ^{13}C)	4 M, 4 F	

From Fabian & Landsiedel (2008)
F, female; M, male

Table 2. Pharmacokinetic parameters of ^{14}C-labelled ametoctradin administered to rats

Sex	Dose (mg/kg bw)	C_{max} (µg Eq/g)	T_{max} (h)	Half-life initial (h)	Half-life intermediate (h)	Half-life terminal (h)	AUC (µg Eq·h/g)
Male	20	0.83	1	2.13	7.71	20.67	6.5
	100	2.43	1	2.42	7.51	31.33	23.0
	500	6.54	1	2.91	8.71	31.21	66.9
	1000	12.53	1	2.54	10.18	29.12	136.4
Female	20	0.73	1	2.51	—	20.67	5.0
	100	2.86	1	1.74	9.21	29.13	22.0
	500	10.32	1	n.r.[a]	8.58	29.88	79.9
	1000	13.10	1	1.91	11.03	28.53	126.1

From Fabian & Landsiedel (2008)
AUC, area under the plasma concentration–time curve; C_{max}, maximum concentration in plasma; n.r., not reliable; T_{max}, time to reach C_{max}
[a] The value of 1.18 given by the study author is considered not reliable, as the plasma value at 1 hour after dosing is not a maximum; the plasma value at 2 hours after dosing is the actual maximum.

Table 3. Recovery of radioactivity in rats up to 168 hours after oral exposure to ametoctradin

Sample	Time interval (h)	Recovery of radioactivity (% of administered dose)					
		50 mg/kg bw single dose		500 mg/kg bw single dose		(14× + 1) × 500 mg/kg bw per day repeated dose	
		M	F	M	F	M	F
Faeces	0–24	57.48	62.21	91.40	76.00	76.44	64.97
	24–48	14.42	19.91	7.02	23.02	17.72	17.46
	48–72	1.04	1.39	1.39	1.03	1.29	0.93
	72–96	0.21	0.27	0.18	0.17	1.05	0.13
	96–120	0.04	0.11	1.84	0.08	0.05	0.96
	120–144	0.03	0.12	0.06	0.06	1.69	0.06
	144–168	0.23	0.05	0.04	0.05	0.04	0.06
	0–168[a]	73.44	84.05	101.91	100.41	98.28	84.56
Urine	0–6	9.82	8.87	3.24	4.05	1.99	2.01
	6–12	4.23	4.95	0.93	0.96	0.88	0.20
	12–24	3.30	4.57	1.16	0.99	0.84	1.83
	24–48	1.66	2.31	0.88	2.01	0.76	1.57
	48–72	0.21	0.56	0.16	0.31	0.15	0.19
	72–96	0.06	0.22	0.09	0.10	0.06	0.12
	96–120	0.05	0.14	0.05	0.04	0.03	0.05
	120–144	0.04	0.11	0.03	0.03	0.02	0.04
	144–168	0.02	0.09	0.01	0.02	0.02	0.04
	0–168[a]	19.37	21.81	6.51	8.51	4.76	6.03
Cage wash	—	0.09	0.35	0.16	0.10	0.14	0.36
Tissue[b]	—	0.12	0.07	0.53	0.43	0.29	0.28
Carcass	—	0.06	0.08	0.31	0.29	0.23	0.27
Total recovery	0–168	93.18	106.36	109.40	109.73	103.72	91.49

From Fabian & Landsiedel (2008)
F, female; M, male
[a] Numbers may not add exactly due to rounding.
[b] Tissues including skin and blood.

Tissue distribution study: Groups of 12 male and 12 female rats were administered a single oral dose of 50 or 500 mg/kg bw. Three animals of each sex were killed at four different time points based on the determined maximum plasma concentrations—namely, C_{max}, ½ C_{max}, ¼ C_{max} and ⅛ C_{max} (see Table 1).

After a single oral dose, ametoctradin was widely distributed, with the highest mean tissue concentrations of radioactivity for the low-dose group of males occurring after 1–2.5 hours in the stomach and stomach contents (174.8–801.0 µg Eq/g) and gut and gut contents (78.9–706.9 µg Eq/g). For the non-gastrointestinal tract tissues, the highest levels were found in the liver (33.4 µg Eq/g) and kidneys (15.2 µg Eq/g). In low-dose females, the highest mean tissue concentrations were found after 1–2.5 hours in the same tissues at similar concentrations. For the males in the high-dose group, the highest mean tissue concentrations were found after 1–4 hours, also in the stomach and stomach contents (1198.6–19 352.5 µg Eq/g), gut and gut contents (31.3–202.3 µg Eq/g), thyroid (64.3 µg Eq/g), liver (31.3 µg Eq/g) and kidneys (22.4 µg Eq/g). In high-dose females, the highest mean tissue concentrations were found after 1–4 hours in the same tissues.

Biliary excretion study: Groups of bile duct–cannulated Wistar rats (four of each sex) received a single oral dose of 50 or 500 mg/kg bw. Bile was collected at 3-hour intervals and urine and faeces were collected at 24-hour intervals for up to 72 hours. The gastrointestinal tract was collected at termination.

Biliary excretion was lower in females than in males in both dose groups, with 12.4% and 22.5% of the administered dose recovered in low-dose females and low-dose males, respectively, and 3.2% and 10.9% in high-dose females and high-dose males, respectively. Biliary excretion took place mainly during the first 48 hours and gradually decreased thereafter. Based on the amount of radioactivity excreted via bile and urine and the radioactivity in cage wash and carcass, the bioavailability of radiolabelled ametoctradin was calculated to be about 36% and 42% in low-dose males and low-dose females, respectively, and about 23% and 16% in high-dose males and high-dose females, respectively (Fabian & Landsiedel, 2008).

Dermal absorption study: No dermal absorption study on the pure active substance is available. The dermal absorption of ametoctradin (purity 99.1%) was studied in vitro in human and rat skin membranes using BAS 651 00 F, a suspension concentrate formulation containing ametoctradin at 300 g/l and dimethomorph at 225 g/l. Ametoctradin was applied at concentrations of 2948 and 1.8 µg active substance per square metre under semi-occluded dressing for 24 hours. As increasing levels of radiolabel were found in receptor fluid from 1 to 24 hours and as ametoctradin is lipophilic, the amount of radiolabel retained in the skin and tape strips should be included to estimate the potential dermal absorption. After 24 hours, 1% and 5% of the applied dose were absorbed following application of the high and low concentrations to rat skin, respectively. For human skin, the corresponding percentages were 0.5% and 3% for the high and low doses, respectively (Gamer, Fabian & Landsiedel, 2007).

1.2 Metabolism

The metabolic fate of ^{14}C-labelled ametoctradin was investigated in a follow-up study. Urine, faeces and bile samples originating from the biokinetics study (Fabian & Landsiedel, 2008) were used to investigate metabolite patterns. Three additional groups of animals were dosed with ^{14}C-labelled ametoctradin specifically for the metabolism study. A summary of the study design is given in Table 4. In dose group DX, urine and faeces were collected at 24-hour intervals over 4 days for female rats and 7 days for male rats. In dose groups V and W, animals were sacrificed at 1 hour post-dosing, and plasma, liver and kidney samples were collected. Urine and bile, extracts of faeces, liver and kidney tissues and plasma were analysed by high-performance liquid chromatography.

Table 4. Study design for metabolism study with ^{14}C-labelled ametoctradin administered to rats

Test group	Dose of labelled material (mg/kg bw)	No. of each sex	Determination
Group B (Experiment 6, Table 1)	50 (^{14}C and ^{13}C)	4	See Table 1
Group C (Experiment 7, Table 1)	500 (14× unlabelled followed by ^{14}C-labelled)	4	
Group D (Experiment 5, Table 1)	500 (^{14}C)	4	
Group R (Experiment 11, Table 1)	50 (^{14}C and ^{13}C)	4	See Table 1
Group S (Experiment 10, Table 1)	500 (^{14}C and ^{13}C)	4	
Group DX	500 (male) / 250 (female) (^{14}C and ^{13}C)	10	Urine and faeces collected at 24 h intervals
Group V	50 (^{14}C and ^{13}C)	4	Liver, kidney and plasma collected after 1 h (C_{max})
Group W	500 (^{14}C and ^{13}C)	4	

From Hafemann & Kloeppner (2008)

Three metabolites, which were generated by full oxidation of the octyl side-chain with subsequent degradation of C-2 or C-1 units, were identified in urine. The ω-hetarylhexanoic acid M650F06 was the most abundant metabolite (5–8% of the administered dose at the low dose and 2–8% at the high dose), followed by the ω-hetarylbutanoic acid M650F01 (0.5–1% at the low dose and 0.3–1% at the high dose) and the ω-hetarylpentanoic acid M650F05 (0.2% at the low dose and 0.05–0.2% at the high dose). In faeces, the excreted radioactivity consisted primarily of the unchanged parent compound (43–69% for the low-dose group, 65–92% for the high-dose group). As in urine, the main metabolites in faeces were M650F06 (11–20% at the low dose and 4–18% at the high dose) and M650F01 (2–5% at the low dose and 0–3% at the high dose). No clear sex-specific differences were observed in either urine or faeces. In bile, the major metabolite in both dose groups and sexes was M650F06 (8–13% at the low dose and 2–5% at the high dose). In addition, two metabolites derived from metabolite M650F06 by conjugation with taurine (M650F12; 1–4% at the low dose and < 0.1–2% at the high dose) or glucuronic acid (M650F11; 0.2–1% at the low dose and < 0.1–1% at the high dose) were identified. Moreover, non-conjugated metabolites were identified as M650F01 (1–3% at the low dose and 0.2–1% at the high dose) and the ω-hetaryloctanoic acid (M650F09; 0.7–0.8% at the low dose and 0–0.2% at the high dose). Another metabolite deriving from metabolite M650F09 by conjugation with taurine was identified to be M650F10. This metabolite was observed only in male rats (3% at the low dose and 2% at the high dose). Overall, the total quantity of metabolites in bile was greater in males than in females. In liver and plasma, M650F06, M650F09 and parent compound were identified. In kidney, the metabolite patterns were comparable to those in liver and plasma, although parent compound was not detected. The total amount of parent compound and metabolites found in liver, kidney and plasma was less than 1% of the administered dose. The proposed metabolic pathway of ametoctradin in the rat is shown in Figure 2 (Hafemann & Kloeppner, 2008).

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

The acute toxicity of ametoctradin is summarized in Table 5. Ametoctradin has low acute toxicity in rats via the oral, dermal and inhalation routes. No substance-related clinical signs were observed after oral or dermal administration. In the inhalation studies, clinical signs of toxicity included visually increased respiration, squatting posture, piloerection, and smeared and contaminated fur.

(b) Dermal irritation

In a dermal irritation study, three male New Zealand White rabbits were exposed to 0.5 g ametoctradin (purity 99.3%, batch COD-000748) applied to a 6.25 cm^2 area of intact skin of the flank under semi-occluded dressing for 4 hours. Skin reactions were scored at 1, 2, 24, 48 and 72 hours following patch removal. Slight erythema was observed in all animals immediately after removal of the patch and after 1 hour. In two out of three animals, the slight erythema was still visible after 24 hours. The cutaneous reactions were reversible in all animals within 48 hours after removal of the patch. No signs of oedema were observed during the study. The individual average scores (24–72 hours) for erythema of each rabbit were 0.3, 0.0 and 0.3, and for oedema, 0.0. It was concluded that ametoctradin does not show a skin irritation potential (Remmele & Landsiedel, 2007a).

In a second dermal irritation study, three male New Zealand White rabbits were cutaneously exposed to 0.5 g ametoctradin (purity 100%, batch F15101209/022) applied to a 6.25 cm^2 area of intact skin of the flank under semi-occluded dressing for 4 hours. Skin reactions were scored at 1, 2, 24, 48, 72 and 96 hours following patch removal. Slight erythema was observed in all animals immediately after removal of the patch. The skin reactions were reversible in all animals within 1 hour after patch removal. The individual average scores (24–72 hours) for erythema as well as oedema of each rabbit were in all cases 0.0. It was concluded that ametoctradin is not a skin irritant (Cords & Lammer, 2010d).

Figure 2. Proposed metabolic pathway of ametoctradin in rat

BAS 650 F

M650F09

M650F10

M650F06

M650F12

M650F11
(or isomer)

M650F05

M650F01

-----▶ minor biotransformation reaction

Table 5. Acute toxicity of ametoctradin

Species	Strain	Sex	Route	Purity; batch	LD_{50}	Reference
Rat	Wistar	Female	Oral	99.3%; COD-000748	> 2000 mg/kg bw	Gamer & Landsiedel (2007a)
Rat	Wistar	Female	Oral	100%; F15101209/022	> 2000 mg/kg bw	Cords (2010a); Cords & Lammer (2010a)
Rat	Wistar	Male and female	Oral	100%; F15101209/022	> 5000 mg/kg bw	Cords & Lammer (2010b)
Rat	Wistar	Male and female	Dermal	99.3%; COD-000748	> 2000 mg/kg bw	Gamer & Landsiedel (2007b)
Rat	Wistar	Male and female	Dermal	100%; F15101209/022	> 2000 mg/kg bw	Cords (2010b); Cords & Lammer (2010c)
Rat	Wistar	Male and female	Inhalation	99.3%; COD-000748	> 5.5 mg/l	Ma-Hock & Landsiedel (2006)
Rat	Wistar	Male and female	Inhalation	100%; F15101209/022	> 5.4 mg/l	Wittmer & Mellert (2010)

LD_{50}, median lethal dose

(c) Ocular irritation

In a study on the eye irritation potential of ametoctradin, 0.1 ml (equivalent to 34 mg) ametoctradin (purity 99.3%, batch COD-000748) was applied into the conjunctival sac of the right eye of three New Zealand White rabbits. The eyes were rinsed with tap water 1 hour after application. Ocular lesions were scored at 1, 24, 48 and 72 hours after application, and signs of irritation were scored according to Draize. No signs of systemic toxicity or mortality were observed during the study period. Slight or moderate conjunctival redness and slight discharge were observed in all three animals at the 1-hour observation period. The ocular reactions were reversible in two animals within 24 hours and in one animal within 48 hours after application. Mean scores for each animal at the 24-, 48- and 72-hour observations were 0.0 for corneal opacity, iris lesions and chemosis and 0.0, 0.0 and 0.3 for conjunctival redness. It was concluded that ametoctradin is non-irritating to eyes (Remmele & Landsiedel, 2007b).

Three adult female New Zealand White rabbits were exposed to 0.1 ml (about 34 mg) ametoctradin (purity 99.3%, batch COD-000748) applied into the conjunctival sac of the right eye. The eyes were rinsed with tap water 24 hours after application, and ocular lesions were scored at 1, 24, 48 and 72 hours. At 1 hour, ocular reactions comprised slight or moderate conjunctival redness, slight conjunctival chemosis and slight discharge. The ocular reactions were reversible in all animals within 24 hours after application. The average scores (24–72 hours) for each rabbit were 0.0 for corneal opacity, iris lesions, conjunctival redness and chemosis. It was concluded that ametoctradin was not an eye irritant (Remmele & Landsiedel, 2007c).

In an eye irritation study, 0.1 ml (about 33 mg) of ametoctradin (purity 100%, batch F15101209/022) was applied into the conjunctival sac of the right eye of three adult female New Zealand White rabbits. About 24 hours after application, the eyes were rinsed with tap water. The rabbits in this study were observed for 7 days following treatment. The ocular reactions were assessed at 1, 24, 48, 72 and 96 hours after application as well as on study day 7. Ocular reactions included slight conjunctival redness in all animals 1 hour after application and persisted in one out of three animals up to 48 hours, in the second animal up to 72 hours and in the third animal up to 96 hours. Slight conjunctival chemosis was noted in one animal 1 hour after application up to 24 hours. The ocular reactions were reversible in all animals within 7 days. Mean scores calculated for each animal over 24, 48 and 72 hours were 0.0 for all animals for corneal opacity and iris lesions, 1.0, 1.0 and 0.7 for redness of the conjunctiva and 0.3, 0.0 and 0.0 for chemosis. It was concluded that ametoctradin is non-irritating to eyes (Cords & Lammer, 2010e).

(d) Dermal sensitization

The skin sensitization potential of ametoctradin (purity 99.3%, batch COD-000748) was investigated using the murine local lymph node assay. Groups of six female CBA/Ca mice were treated with 10%, 30% or 50% weight per weight (w/w) preparations. Higher concentrations were not technically achievable. The vehicle was propylene glycol. For 3 consecutive days, 25 µl of the respective test substance or vehicle control alone was applied to the dorsum of both ears. On day 5, the mice received 0.74 MBq of ^{3}H-labelled thymidine in 250 µl of sterile saline by intravenous injection into a tail vein. About 5 hours after ^{3}H-labelled thymidine treatment, the mice were sacrificed, and the auricular lymph nodes were removed. From each animal, ear thickness, ear weight and lymph node weight were determined, in addition to measurement of ^{3}H-labelled thymidine incorporation into the lymph node cells. No signs of systemic toxicity were noticed. No increase in stimulation index (SI) of cell count by a factor of greater than 1.5 or of ^{3}H-labelled thymidine incorporation by a factor of greater than or equal to 3 was observed after ametoctradin exposure. It was concluded that ametoctradin, at concentrations up to 50%, does not show a skin sensitizing effect in the murine local lymph node assay (Gamer & Landsiedel, 2007c).

In a second murine local lymph node assay, groups of five female CBA/J mice were treated with either 50% w/w preparations of ametoctradin (purity 100%, batch F15101209/022) or the vehicle alone. Higher concentrations were not technically achievable. The vehicle was a mixture of acetone and olive oil. A group of five female CBA/J mice treated with 25% α-hexylcinnamaldehyde in the

acetone/olive oil vehicle was included as a positive control. The respective test substance preparation, vehicle control or positive control (25 µl per ear) was applied to the dorsum of both ears of each test group animal for 3 consecutive days. On day 5, the mice received 0.74 MBq of ^{3}H-labelled thymidine in 250 µl of sterile saline by intravenous injection into a tail vein. The animals were sacrificed about 5 hours after ^{3}H-labelled thymidine injection. From each animal, ear thickness, ear weight and lymph node weight were determined, in addition to measurement of ^{3}H-labelled thymidine incorporation into lymph node cells. No signs of systemic toxicity were observed. When applied as a 50% preparation in acetone/olive oil vehicle, no increase in SI by a factor of greater than 1.5 or of ^{3}H-labelled thymidine incorporation by a factor of greater than or equal to 3 was observed. It was concluded that ametoctradin, in a 50% preparation, is not a skin sensitizer (Remmele & Landsiedel, 2010).

In a dermal sensitization study using the Magnusson and Kligman maximization test, ametoctradin (purity 99.3%, batch COD-000748) was tested using 10 Dunkin-Hartley guinea-pigs. The control group consisted of five animals. The intradermal induction step was performed on day 0 with a 5% test substance preparation in 1% aqueous carboxymethylcellulose solution or a 5% test substance preparation in Freund's complete adjuvant/0.9% aqueous sodium chloride solution (1:1). The topical induction was performed on day 7 with a 60% test substance preparation in 1% aqueous carboxymethylcellulose solution (highest concentration technically achievable). For the topical challenge, on day 21, the 60% test substance preparation in 1% aqueous carboxymethylcellulose solution was applied. The intradermal induction caused moderate and confluent to intense erythema. Moreover, swelling was observed at the injection sites in all test group animals. After topical induction, incrustation (partially open) could be observed, in addition to moderate and confluent erythema and swelling in all test group animals. After topical challenge, discrete or patchy erythema was noted in two test group animals after 24 hours. No skin findings were observed in any of the test group animals 48 hours after removal of the patch. It was concluded that ametoctradin does not have a sensitizing effect on the skin in the maximization test (Gamer & Landsiedel, 2007d; Gamer, 2009).

2.2 *Short-term studies of toxicity*

(a) Oral administration

Mice

In a 90-day feeding study in C57BL/6NCrl mice, ametoctradin (purity 99.8%, batch COD-000567) was administered to groups of mice (10 of each sex) at a dietary concentration of 0, 500, 2000 or 6000 parts per million (ppm) (equal to 0, 100.8, 370.3 and 1118.8 mg/kg bw per day for males and 0, 167.6, 596.6 and 2086.5 mg/kg bw per day for females, respectively). Feed consumption and body weight were determined weekly. Clinical observation was undertaken at least once a day. Clinical biochemical and haematological examinations were performed towards the end of the administration period. Cell proliferation (S-phase response) was evaluated in the liver, kidney and urinary bladder using osmotic minipumps with 5-bromo-2′-deoxyuridine (BrdU) implanted in all animals 7 days before necropsy.

The mortality incidence, clinical signs, feed consumption and body weight were not affected after dietary exposure to ametoctradin. No treatment-related changes were observed in haematological and clinical chemistry parameters, organ weights, pathological findings or cell proliferation in liver, kidney and urinary bladder.

The no-observed-adverse-effect level (NOAEL) was 6000 ppm (equal to 1118.8 mg/kg bw per day for males and 2086.5 mg/kg bw per day for females), the highest dose tested (Kaspers et al., 2007b).

Rats

In a short-term study of toxicity, Wistar rats (10 of each sex per group) were dosed with ametoctradin (purity 99.8%, batch COD-000567) for at least 90 days at a dietary concentration of 0, 1500, 5000 or 15 000 ppm (equal to 0, 105.8, 358.3 and 1083.2 mg/kg bw per day for males and 0,

123.3, 415.8 and 1235.1 mg/kg bw per day for females, respectively). Rats were observed for clinical signs at least once a day, whereas body weight and feed consumption were determined weekly. Ophthalmoscopic observations were carried out once prior to treatment and on day 77. Neurobehavioural screening, consisting of functional observational battery and motor activity examinations, was performed at the end of the study. Clinical biochemistry and haematological examinations were performed towards the end of the administration period. At necropsy, the weights of selected organs were recorded, and the organs were assessed by gross examination and examined histopathologically. Seven days prior to necropsy, osmotic minipumps subcutaneously filled with BrdU were implanted for S-phase response determination in the liver, kidney, urinary bladder and thyroid. Labelling indices were determined in control and high-dose animals.

There were no treatment-related changes in mortality, clinical signs of toxicity, body weight, feed consumption, ophthalmoscopic examination, neurobehavioural end-points, haematological parameters and urine analysis throughout the study at any dose level. In addition, there were no substance-related effects on the S-phase response in the tissues examined. For the blood chemistry analysis, a non-significant increase in triglycerides was noted for males in the high-dose group (144% of control), which was within the historical control range and therefore considered to be unrelated to treatment. In high-dose males, there was a slight increase in liver weight by 10% (statistically significant relative to body weight). Because there were no corresponding histopathological changes found in the liver, this increase in liver weight is considered to be not treatment related or toxicologically relevant.

The NOAEL was 15 000 ppm (equal to 1083.2 mg/kg bw per day for males and 1235.1 mg/kg bw per day for females), the highest dose tested (Kaspers et al., 2007a).

Dogs

In a 90-day dietary study, groups of purebred Beagle dogs (five of each sex per dose) were dosed with ametoctradin (purity 99.3%, batch COD-000748) at a dietary concentration of 0, 3000, 10 000 or 30 000 ppm (equal to 0, 93, 299 and 912 mg/kg bw per day for males and 0, 100, 330 and 1006 mg/kg bw per day for females, respectively). Feed consumption was determined each working day, and body weight once a week. At least once a day, animals were observed for signs of toxicity. Ophthalmoscopic examinations were carried out 13 days prior to treatment and at the end of the study period. Clinical chemistry, haematological examinations and urine analyses were carried once before treatment, after 6 weeks and towards the end of the substance administration. After necropsy, all animals were subjected to gross pathological assessment, followed by histopathological examination.

No mortality or changes in feed consumption or body weight were noted. Regarding clinical observation, ophthalmoscopy, clinical chemistry, haematological examination and urine analyses, dietary exposure to ametoctradin at concentrations up to 30 000 ppm did not induce any treatment-related changes. A statistically significant increase in absolute thyroid weight was noted at 10 000 and 30 000 ppm in females (28.7% and 26.3%, respectively). In addition, a non-significant decrease in mean uterine weight was found in females at the high dose (37.8%). As there were no corresponding histological findings, these changes in organ weights were regarded to be incidental and not related to the test substance.

The NOAEL was 30 000 ppm (equal to 912 mg/kg bw per day for males and 1006 mg/kg bw per day for females), the highest dose tested (Hempel et al., 2007).

In a 1-year dietary toxicity study, ametoctradin (purity 99.3%, batch COD-000748) was administered to Beagle dogs (five of each sex per dose) at 0, 3000, 10 000 or 30 000 ppm (equal to 0, 84, 273 and 848 mg/kg bw per day for males and 0, 85, 305 and 936 mg/kg bw per day for females, respectively). The dogs were examined at least once a day for signs of toxicity. Feed consumption was determined each working day, and body weight on a weekly basis. Ophthalmoscopic examinations were performed once before the start and at the end of the study. Haematological and clinical chemistry parameters were determined at the end of the study period. Urine analyses were

carried out before the start of treatment and after about 3, 6 and 12 months. After necropsy, selected organs and tissues were examined by gross pathology and histopathology.

No substance-related mortality was observed upon dietary exposure to ametoctradin for 1 year. Although decreased feed intake was not observed on all days on which it was determined, overall, during the whole study period, feed intake was lower in low-dose females than in controls. In addition, for mid-dose males, a lower feed intake was noted on several occasions during the exposure period. Body weight gain was increased in mid-dose females on several occasions. As no significant effect on body weight was observed, these effects were considered not to be toxicologically relevant. Ophthalmoscopic examinations revealed no substance-related findings. White blood cell count was statistically significantly reduced in mid-dose males only. Tubular degeneration of the testes and epididymides (aspermia and oligospermia) was noted in high-dose males. However, the effects on the epididymides were not observed in the high-dose males in the 90-day dietary toxicity study (see above), but were observed in one male of the control and intermediate-dose groups. Tubular degeneration was observed in the 90-day dog study (see above) in one male of each dose group, but in a comparison of the severity, there was no dose–response relationship. This indicates that the findings in the testes and epididymides can be considered incidental and not test substance related.

The NOAEL was 30 000 ppm (equal to 848 mg/kg bw per day for males and 936 mg/kg bw per day for females), the highest dose tested (Hempel et al., 2008).

(b) Dermal application

In a 28-day dermal toxicity study, ametoctradin (purity 99.3%, batch COD-000748) was applied to the shaved, intact dorsal skin of Wistar rats (10 of each sex per dose) at a dose of 0, 100, 300 or 1000 mg/kg bw per day under semi-occlusion. The vehicle was 1% aqueous carboxymethylcellulose. Animals were examined at least once a day for signs of toxicity. Body weight and feed consumption were determined on a weekly basis. Ophthalmoscopy was carried out once prior to the start of the study and once at the end of the administration period. A functional observational battery was performed on all animals at the end of the study. Urine analyses, clinical biochemistry as well as haematological examinations were carried out at the end of the study. All animals underwent gross pathological and histopathological examinations.

No substance-related effects on mortality, clinical signs, feed consumption or ophthalmological examinations were observed. The neurobehavioural studies did not reveal any treatment-related effects. Males in the mid- and high-dose groups showed a lower body weight gain on days 21 and 28, resulting in slightly lower body weights on those days (approximately 5% lower). Haematological and urine analyses parameters were unchanged. Clinical biochemistry parameters showed an increase in bilirubin levels in males at 100, 300 and 1000 mg/kg bw per day (109%, 113% and 121% of controls, respectively). As these changes were not accompanied by other corroborative changes (e.g. changes in red blood cells), this finding was not considered to be toxicologically relevant. The absolute weight of the brain in high-dose males was statistically significantly decreased (4%) compared with controls. The statistically significant change in absolute brain weight is regarded as not treatment related, as no relative weight changes or histopathological findings were noted in the brain. The relative weights of the adrenal glands in the males at 100 and 1000 mg/kg bw per day and the relative weight of the liver in the males at 300 mg/kg bw per day were statistically significantly increased (18.2% in both dose groups for relative adrenal gland weight and 4.9% for relative liver weight). As the effects were without a dose–response relationship and the organs showed no corresponding histopathological changes, the findings were not considered to be toxicologically relevant.

The NOAEL in both males and females was 1000 mg/kg bw per day, the highest dose tested (Kaspers et al., 2007c; Kaspers, 2009).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a carcinogenicity study, C57BL/6 J Rj mice (50 of each sex per group) received ametoctradin (purity 99.3%, batch COD-000748) at a dietary concentration of 0, 60, 600 or 6000 ppm (equal to 0, 10.6, 104.2 and 1099 mg/kg bw per day for males and 0, 15.2, 154.1 and 1543 mg/kg bw per day for females) for 18 months. Observations for mortality and clinical signs were carried out at least once a day. Feed consumption and body weight were monitored weekly during the first 13 weeks and thereafter at 4-week intervals until the end of the administration period. Haematological and clinical chemistry examinations were performed at the end of the study. At necropsy, the weights of selected organs were recorded for all mice. All major organs were examined by gross microscopy as well as histopathologically.

No substance-related changes in mortality, clinical signs or feed consumption were noted. A slight, but statistically significant, decrease in body weight (gain) was observed in high-dose males only. As the decrease in body weight was less than 10%, this effect was not considered to be toxicologically relevant. At the end of dosing, a slightly higher incidence of different plasma changes in the lymphocytes of the high-dose females was observed (8/47 mice compared with 3/46 mice in the controls). However, the counts of changed lymphocytes per dosed mouse were between 1 and 2 per 100 cells, whereas 2–6 changed lymphocytes per 100 cells were found in control mice. Furthermore, the relevance of these haematological effects in such old mice, with a high standard deviation, can be questioned. At the end of the administration period, slightly more females in the 6000 ppm dose group with 1–2 metamyelocytes per 100 cells were found (5/47 mice compared with 0/46 mice in the controls). However, as a few mice with single metamyelocytes were also found in the controls of both sexes after 12 months as well as in male animals at the end of the administration period, the effect can be regarded as incidental and not substance related. Both the absolute and relative kidney weights were significantly reduced (−8.8% and −8.2%, respectively) in high-dose males. In high-dose females, absolute liver weight was increased by 11% compared with controls. As the observed weight changes were only slight and not accompanied by other toxic effects, they were not considered to be toxicologically relevant. There were no treatment-related effects on the incidence of neoplastic or non-neoplastic lesions.

Based on the absence of toxicologically relevant effects in the dose groups tested, the NOAEL was set at 6000 ppm (equal to 1099 mg/kg bw per day for males and 1543 mg/kg bw per day for females), the highest dose tested. Ametoctradin showed no carcinogenic potential in mice (Kamp et al., 2008).

Rats

In a 2-year combined chronic toxicity and carcinogenicity study, ametoctradin (purity 99.1–99.3%, batches COD-000682 and COD-000748) was administered to Wistar rats (50 of each sex per group). Dietary concentrations of 0, 150, 1500 and 15 000 ppm (raised to 20 000 ppm on day 308 and to 22 500 ppm on day 336 in males) were used, equal to a daily test substance intake over the entire administration period of 0, 6.9, 69.9 and 870.7 mg/kg bw per day for males and 0, 9.6, 95.0 and 979.3 mg/kg bw per day for females, respectively. As the test substance concentration in feed was raised twice for high-dose males during the study, the intakes at the beginning and end of the study and around the increase on days 308 and 336 are indicated in Table 6.

Additional groups of rats (10 of each sex per dose) were treated with ametoctradin for interim sacrifice after 1 year. Feed consumption and body weight were observed weekly for the first 13 weeks and at 4-week intervals thereafter. The animals were examined for signs of toxicity or mortality at least once a day. An ophthalmoscopic examination was carried out on all animals prior to treatment and at the end of the study. Urine analysis, clinical chemistry and haematological examinations were carried out in the interim sacrifice group at 3, 6 and 12 months. After necropsy, selected organs were weighed, and complete macroscopic and histopathological evaluations were conducted on all animals.

Table 6. Mean intake of ametoctradin in male rats in the 2-year study

Day	Mean test substance intake (mg/kg bw per day)		
	150 ppm	1500 ppm	15 000 ppm
7	14.5	144.0	1459.3
287	6.5	66.5	664.4
315[a]	6.3	64.2	855.9
336[a]	NC	NC	858.3
343	6.3	62.9	963.5
728	5.6	59.2	899.2

From Kaspers et al. (2008a); Buesen (2010a,b)

NC, not calculable

[a] Dietary concentration for high dose group males was raised from 15 000 to 20 000 ppm on day 308 and to 22 500 ppm on day 336.

No substance-related mortality or clinical signs of toxicity were observed during dietary exposure of the rats to ametoctradin. Body weight was significantly lower in high-dose males from day 357 (approximately 4%) until the end of the study (approximately 8%). Similarly, body weight gain was also significantly reduced. In high-dose females, significant decreases in body weight (maximally 7%) and body weight gain were noted throughout the study. However, no effect on feed consumption was observed in either sex. As the decreases in both body weight and body weight gain at the end of the study were small (< 10%), they were not considered toxicologically relevant. No substance-related effects were observed in ophthalmology, clinical chemistry, urine analysis or haematology. In males, an increase in mean absolute epididymides weight was observed in the satellite group at all doses (21.7%, 8.8% and 9.0% in low-, mid- and high-dose males, respectively), and an increase in relative epididymides weight was observed in the main group (13.2%, 13.7% and 10.6%, respectively). In addition, increases in relative kidney weight in all dosed males (14.2%, 10.8% and 8.1% in low-, mid- and high-dose males, respectively), in relative heart weight in low- and mid-dose males (10% and 6.3%, respectively) and in relative liver weight in mid- and high-dose males (4.8% and 7.2%, respectively) were noted. However, the weight increases were not dose related, were observed only in males and were not accompanied by histopathological findings. An increase in the incidence of dilution of the ductus choledochus was observed at 24 months in mid- and high-dose males (25.6% and 26.5% compared with 15.8% in controls) and in high-dose females (20% compared with 8.8% in controls). In the absence of corroborative findings in haematological parameters, clinical signs and histopathology, the effect was not considered to be adverse. No other substance-related non-neoplastic findings were observed.

There were no substance-related neoplastic findings.

The NOAEL was concluded to be 870.7 mg/kg bw per day for males and 979.3 mg/kg bw per day for females, the highest dose tested in both sexes. Ametoctradin was not carcinogenic in Wistar rats under the study conditions (Kaspers et al., 2008a; Buesen, 2010a,b).

2.4 Genotoxicity

The genotoxic potential of ametoctradin was evaluated in a battery of four in vitro and four in vivo studies (Table 7). Ametoctradin gave negative results in all these studies. Therefore, ametoctradin is unlikely to be genotoxic.

Table 7. Results of genotoxicity studies with ametoctradin

End-point	Test system	Concentration/dose	Purity; batch	Result	Reference
In vitro					
Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537; *Escherichia coli* WP2*uvrA*; standard plate incorporation and preincubation assay	20–5000 µg/plate (±S9)	99.3%; COD-00748	Negative	Schulz & Landsiedel (2006)
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537; *E. coli* WP2*uvrA*; standard plate incorporation and preincubation assay	33–5000 µg/plate (±S9)	100%; F15101209/022	Negative	Schulz & Landsiedel (2010a)
Chromosomal aberrations	V79 cells	−S9: 25–100 µg/ml (4 h) 25–200 µg/ml (18 h) +S9: 12.5–100 µg/ml (4 h)	99.5%; COD-000480	Negative	Schulz (2005)
Gene mutation (HPRT)	CHO-K1 cells	1st experiment: 31.3–1000 µg/ml (±S9) 2nd experiment: 25–300 µg/ml (±S9)	99.3%; COD-000748	Negative	Schulz & Landsiedel (2007a)
In vivo					
Chromosomal aberrations	Wistar rats, bone marrow cells	500–2000 mg/kg bw	99.5%; COD-000480	Negative	Engelhardt & Leibold (2005a); Landsiedel (2008)
Micronucleus formation	NMRI mice, bone marrow cells	500–2000 mg/kg bw	99.8%; COD-000567	Negative	Engelhardt & Leibold (2005b)
Micronucleus formation	NMRI mice, bone marrow cells	2000 mg/kg bw	100%; F15101209/022	Negative	Schulz & Landsiedel (2010b)
Unscheduled DNA synthesis	Wistar rats, primary hepatocytes	1000, 2000 mg/kg bw	99.5%; COD-000480	Negative	Honarvar (2005)

DNA, deoxyribonucleic acid; S9, 9000 × *g* supernatant fraction of rat liver homogenate

2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

In a two-generation reproductive toxicity study, ametoctradin (purity 99.3%, batch COD-00748) was administered to groups of 25 male and 25 female Wistar rats continuously throughout the study at dietary concentrations that were adjusted to obtain target dose levels of 0, 100, 300 and 1000

mg/kg bw per day. The actual dietary intakes for the F_0 and F_1 generations are shown in Table 8. At least 75 days after the beginning of treatment, F_0 animals were mated to form the F_1 generation. Groups of 25 males and 25 females, selected from F_1 pups to become the F_1 parental generation, were continued on the same dose as their parents post-weaning, and the breeding programme was repeated to produce an F_2 litter. The examinations of parental animals included monitoring of mortality, clinical signs, feed consumption and body weight. The pups were weighed on postnatal days 1, 4, 7, 14 and 21. A comprehensive evaluation of male and female reproductive systems was conducted, including evaluation of the estrous cycle, mating performance, sperm parameters, conception, gestation, parturition and lactation, as well as survival, growth and development of the offspring. All F_0 and F_1 parental animals were assessed for changes in organ weights, gross pathology and histopathology. After termination, all pups were examined externally and their organs were assessed macroscopically. The brain, spleen and thymus of one pup of each sex and litter from the F_1 and F_2 generations were weighed.

Table 8. Mean intake of ametoctradin at target dose levels in the two-generation study in rats

	Average ametoctradin intake (mg/kg bw per day)					
	F_0 generation			F_1 generation		
	100 mg/kg bw per day	300 mg/kg bw per day	1000 mg/kg bw per day	100 mg/kg bw per day	300 mg/kg bw per day	1000 mg/kg bw per day
Males	94.4	282.6	944.0	93.6	280.2	938.7
Females, premating	95.5	285.3	951.3	96.8	291.3	965.3
Females, gestation period	101.2	308.7	1044.9	100.8	297.6	1043.0
Females, lactation period	95.8	296.7	1010.9	93.7	285.3	954.9

In F_0 and F_1 animals, mortality, feed consumption and body weight were not affected by treatment, and no treatment-related clinical signs were observed. In addition, there were no treatment-related effects on reproductive parameters. Relative liver and kidney weights were significantly increased in F_0 and F_1 animals in all dose groups (maximum increase of 10%). However, owing to the lack of a clear dose–response relationship and the absence of corresponding histopathological findings, these effects were not considered to be toxicologically relevant. In F_0 females, significant decreases in uterine weight (16% and 18%, respectively) were noted for the mid- and high-dose groups. In F_1 females, a similar significant decrease in uterine weight was observed in high-dose animals only. However, a high variation in uterine weight was apparent. Furthermore, the observed weight decreases were within the historical control range and not corroborated by any histopathological findings or functional impairment. In F_1 pups, perinatal and postnatal survival and post-weaning development until sexual maturity were unaffected by the test substance at any dose tested. No substance-related changes were observed in litter size, pup viability, sex ratio or clinical signs of the F_2 pups.

The NOAEL was 939 mg/kg bw per day, the highest dose tested, with regard to systemic toxicity, fertility and reproductive performance in parental F_0 and F_1 rats as well as prenatal and postnatal developmental toxicity in their offspring (Schneider et al., 2008a).

(b) Developmental toxicity

Rats

In a prenatal developmental toxicity study, groups of 25 time-mated female Wistar rats were dosed with ametoctradin (purity 99.8%, batch COD-000567) by gavage at 0, 100, 300 or 1000 mg/kg bw per day on gestation day (GD) 6 to GD 19. Mortality and clinical symptoms were examined at

least once a day. All animals were weighed on GDs 0, 1, 3, 6, 8, 10, 13, 15, 17, 19 and 20. Feed consumption was determined on the same days as body weight, with the exception of GD 0. On GD 20, all females were sacrificed and underwent gross pathological examination. Examinations included uterine weight and numbers of corpora lutea, implantation sites, live and dead fetuses, and resorptions. Each fetus was weighed, sexed and examined macroscopically for any external findings. Approximately half of the fetuses were preserved in Harrison's fluid and examined for any visceral findings. The remaining fetuses were eviscerated, skinned, fixed in ethyl alcohol and then stained with alizarin red and alcian blue for skeletal examination.

There were no substance-related effects on mortality, clinical signs, feed consumption or body weight in the maternal animals. The number of corpora lutea, the number of viable fetuses and fetal weight were unaffected by ametoctradin at all dose levels. There were no treatment-related changes observed in the external and visceral examinations. However, several skeletal variations were recorded. A significant increase in the incidence of incomplete ossification of the skull was observed at the high dose (9.4% affected fetuses per litter), but it remained within the range of historical controls (0–11%). In high-dose animals, a significant increase in the incidence of misshaped tuberositas deltoidea was also noted (3.8%), but it was within the range of the historical controls (0–6.8%). A significant increase in the incidence of bipartite ossification of thoracic centrum was observed in the mid-dose group only. Considering the lack of a dose–response relationship, this effect was not considered to be toxicologically relevant. The incidence of misshapen sternebrae was also significantly increased at the high dose (53.1% affected fetuses per litter compared with 38.8% in the controls and 7.7–52.1% in the historical controls). Considering the high incidence in control animals, 53.1% is only slightly higher than the upper limit of the historical control range and therefore considered incidental and of minor toxicological relevance.

The NOAEL for maternal toxicity was 1000 mg/kg bw per day. As all the skeletal variations were within the historical control ranges or only marginally above the upper limit of the range, these findings were considered to be incidental. Therefore, the NOAEL for developmental toxicity was set at 1000 mg/kg bw per day, the highest dose tested (Schneider et al., 2006).

Rabbits

Ametoctradin (purity 99.3%, batch COD-000748) was tested for its prenatal developmental toxicity in Himalayan rabbits. Groups of 25 female inseminated Himalayan rabbits were administered the test substance by gavage at a dose of 0, 100, 300 or 1000 mg/kg bw per day from GD 6 through GD 28. Mortality and clinical symptoms were checked on a daily basis. Feed consumption and body weight were observed regularly throughout the study period. On GD 29, the surviving females were sacrificed and assessed by gross pathological examination. For each animal, uterine weight and the numbers of corpora lutea, early and late resorptions, and live and dead fetuses were recorded. The fetuses were removed from the uterus, sexed, weighed and further evaluated for any external, soft tissue and skeletal findings, including cartilage staining.

Ametoctradin showed no effect on mortality or clinical signs. Feed consumption was slightly higher in high-dose females, and consequently a slightly higher final body weight was observed. However, maternal body weights were still within the range of the historical controls. There were no treatment-related effects on the numbers of corpora lutea, preimplantation and post-implantation losses or viable fetuses, sex ratio or fetal weight. In addition, there were no significant changes in external observations in any of the dose groups. Visceral examination showed a slight increase in the incidence of haemorrhagic ovary (1.4% affected fetuses per litter) and in the incidence of blood coagulum around the urinary bladder (2.8% affected fetuses per litter). As the observed incidences were within the range of the historical controls (0–3.8% for haemorrhagic ovary and 0–8.3% for blood coagulum around the urinary bladder), the effects were considered incidental. The incidence of soft tissue variations was increased in the mid-dose group only (15.2% affected fetuses per litter compared with 5.7% in controls), mainly due to an increase in the incidence of absent lung lobe (14% compared with 3.8% in controls). However, the effect was within the same range as the historical control (3.3–33.6%). An increase in the incidence of supernumerary 13th rib was noted at the high dose (7.0%

affected fetuses per litter). However, the observed incidence was within the historical control range (0–10.4%) and therefore considered to be not adverse.

The NOAEL for maternal and prenatal developmental toxicity was 1000 mg/kg bw per day, the highest dose tested. No indications for teratogenicity were observed in any test group (Schneider et al., 2008b).

2.6 *Special studies*

(a) Neurotoxicity

The acute neurotoxicity of ametoctradin (purity 99.3%, batch COD-000748) was investigated in Wistar rats (10 of each sex per group) after a single oral administration by gavage at a dose of 0, 125, 500 or 2000 mg/kg bw. The animals were observed for up to 2 weeks after dosing. General health was observed daily. Body weight, functional observational battery and motor activity measurements were carried out on days −6, 0, 7 and 14. At the end of the study, five animals of each sex per dose were fixed by in situ perfusion and subjected to neuropathological examination.

No treatment-related changes were observed in mortality, clinical symptoms or body weight. In addition, functional observational battery and motor activity measurements were not affected by ametoctradin at doses up to 2000 mg/kg bw, nor were any substance-related neuropathological changes observed.

The NOAEL for acute neurotoxicity was 2000 mg/kg bw, the highest dose tested (Kaspers et al., 2009a).

In a subchronic neurotoxicity study, ametoctradin (purity 99.3%, batch COD-000748) was administered to Wistar rats (10 of each sex per dose) at a dietary concentration of 0, 1500, 5000 or 15 000 ppm (equal to doses of 0, 89.4, 299.9 and 921.2 mg/kg bw per day for males and 0, 104.5, 349.7 and 1077.2 mg/kg bw per day for females, respectively) for a period of 3 months. Mortality and signs of toxicity were examined at least once a day. Feed consumption was determined once a week. Body weight was measured weekly and on days when functional observational battery tests were performed. Functional observational batteries and measurement of motor activity were carried out on days −7, 1, 22, 50 and 85. Five animals of each sex per dose were fixed by in situ perfusion and subjected to neuropathological examination.

Ametoctradin at dietary concentrations up to 15 000 ppm did not affect mortality, clinical examinations or body weight. No changes with regard to the functional observational battery or motor activity measurements were observed. In addition, no treatment-related effects were noted in the neuropathological examinations.

Under the conditions of this study, the NOAEL for neurotoxicity was 15 000 ppm (equal to 921.2 mg/kg bw per day for males and 1077.2 mg/kg bw per day for females), the highest dose tested (Kaspers et al., 2009b).

(b) Immunotoxicity

Ametoctradin (purity 99.3%, batch COD-000748) was administered to groups of eight female C57BL/6 J Rj mice at a dose of 0, 500, 2000 or 6000 ppm (equal to 0, 160.0, 603.5 and 1955.7 mg/kg bw per day, respectively) in the diet over a period of 4 weeks. In addition, a positive control group received cyclophosphamide monohydrate by gavage at a dose of 12 mg/kg bw per day. Feed consumption and body weight were determined weekly. The animals were examined for mortality and signs of toxicity at least once a day. Lymphocyte count, determinations of lymphocyte subpopulations, measurements of sheep red blood cell immunoglobulin M (IgM) antibody titres as well as natural killer (NK) cell activity tests were carried out at the end of the study. In addition, all

animals were assessed by gross pathological examination, and the organ weights of spleen and thymus were measured.

There were no treatment-related effects, and no mortality or clinical signs were observed after ametoctradin treatment. Moreover, feed consumption and body weight were unchanged in animals treated with ametoctradin compared with controls. No changes in total lymphocyte counts or in absolute and relative lymphocyte subpopulation counts were measured in the dosed animals. In addition, no test substance–related changes were observed in NK cell activity, in the sheep red blood cell IgM titres or in the pathological examinations.

The NOAEL was 6000 ppm (equal to 1955.7 mg/kg bw per day), the highest dose tested (Kaspers et al., 2009c).

3. Studies on metabolites

3.1 M650F02

4-(7-Amino-5-ethyl[1,2,4]triazolo[1,5-a]pyrimidin-6-yl)propanoic acid (company code M650F02) was tested in two genotoxicity tests, with negative results (Table 9).

Table 9. Results of genotoxicity studies with M650F02

End-point	Test system	Concentration/ dose	Purity; batch	Result	Reference
In vitro					
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537; *E. coli* WP2*uvrA*; standard plate incorporation and preincubation assay	24–6000 µg/plate (±S9)	87.4%; L71-56	Negative	Engelhardt & Hellwig (2005a); Engelhardt (2006a)
In vivo					
Micronucleus formation	NRMI mice, bone marrow cells	575–2300 mg/kg bw	87.4%; L71-56	Negative	Engelhardt & Mellert (2006)

S9, 9000 × g supernatant fraction of rat liver homogenate

3.2 M650F03

In a 90-day oral toxicity study, Wistar rats (10 of each sex per group) received 7-amino-5-ethyl[1,2,4]triazolo[1,5-a]pyrimidin-6-acetic acid (company code M650F03) (purity 97.2%, batch L71-154) at a dietary concentration of 0, 1500, 5000 or 15 000 ppm (equal to 0, 89.5, 298.8 and 942.6 mg/kg bw per day for males and 0, 107.1, 348.6 and 1093.6 mg/kg bw per day for females, respectively). Feed consumption and body weight were determined weekly. The animals were examined for signs of toxicity or mortality at least once a day. Functional observational battery tests, measurement of motor activity, clinical biochemistry, haematological examinations and urine analyses were performed towards the end of the treatment period. Ophthalmoscopic examinations were conducted before treatment and towards the end of the administration period. All animals underwent gross pathological and histopathological examinations.

No mortalities were observed in any of the dose groups. Body weight, feed consumption, functional observational battery, ophthalmoscopy, haematology, clinical biochemistry and urine analysis were unchanged after exposure to dietary concentrations of ametoctradin up to 15 000 ppm. Regarding motor activity, a slight decrease was noted in the overall beam interrupts (interval 1–12) in high-dose males. As no other effects on motor activity were observed, this slight effect was considered not substance related or toxicologically relevant. No treatment-related adverse effects on clinical pathology parameters were observed.

The NOAEL was 15 000 ppm (equal to 942.6 mg/kg bw per day for males and 1093.6 mg/kg bw per day for females), the highest dose tested (Kaspers et al., 2008b).

M650F03 was tested in a battery of in vitro and in vivo genotoxicity studies (Table 10). M650F03 was negative in all tests.

Table 10. Results of genotoxicity studies with M650F03

End-point	Test system	Concentration/dose	Purity; batch	Result	Reference
In vitro					
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537; *E. coli* WP2*uvrA*; standard plate incorporation (1st experiment) and preincubation assay (2nd and 3rd experiments)	1st experiment: 20–5000 µg/plate (±S9) 2nd experiment: 4–2500 µg/plate (±S9) 3rd experiment: 500–4000 µg/plate (±S9)	98.1%; L71-55	Negative	Engelhardt & Hellwig (2005b); Engelhardt (2006b)
Gene mutation (HPRT)	CHO-K1 cells	1st experiment −S9: 800–1800 µg/ml +S9: 100–1000 µg/ml 2nd experiment: −S9: 1400–1600 µg/ml +S9: 500–1000 µg/ml	98.1%; L71-55	Negative	Engelhardt & Landsiedel (2006)
Chromosomal aberration	V79 cells	1st experiment: −S9: 275–2200 µg/ml (18 h) +S9: 137.5–2200 µg/ml (18 h) 2nd experiment: −S9: 275–2200 µg/ml (18 h) −S9: 1100–2200 µg/ml (28 h) +S9: 550–2200 µg/ml (28 h)	98.1%; L71-55	Negative	Schulz & Landsiedel (2007b)
In vivo					
Micronucleus formation	NMRI mice, bone marrow	500–2000 mg/kg bw	98.1%; L71-55	Negative	Engelhardt (2006c); Engelhardt & Hellwig (2006)

S9, 9000 × *g* supernatant fraction of rat liver homogenate

3.3 M650F04

7-Amino-5-ethyl[1,2,4]triazolo[1,5-a]pyrimidin-6-carboxylic acid (company code M650F04) (purity 95.8%, batch L71-126) was administered to Wistar rats (10 of each sex per group) at a dietary

concentration of 0, 1500, 5000 or 15 000 ppm (equal to 0, 96.9, 317.9 and 1033.5 mg/kg bw per day for males and 0, 114.9, 418.0 and 1161.4 mg/kg bw per day for females, respectively) for 3 months. Feed consumption and body weight were recorded on a weekly basis. The animals were examined for signs of toxicity or mortality at least once a day. A functional observational battery, measurement of motor activity, clinical biochemistry, haematological examinations and urine analyses were carried out towards the end of the study. Ophthalmoscopic examinations were performed before and towards the end of the administration period. After termination, all animals underwent gross pathological and histopathological examinations.

No mortalities were observed in any of the treatment groups throughout the study, nor were any treatment-related clinical signs noted. Increased water consumption was observed in high-dose males (20–50%) and females (10–30%). However, the increase in water consumption was not accompanied by increased urine production. No treatment-related changes were observed in feed consumption, haematology, clinical chemistry, ophthalmoscopy or urine analysis. In motor activity tests, high-dose females showed a slight decrease in the overall beam interrupts (interval 1–12) compared with controls. However, no other effects on motor activity were found. A slight increase in absolute brain weight was observed in low-dose (3%) and high-dose males (2%). The absence of a dose–response relationship and the lack of corroborating histopathological findings suggested that the observed effect was not toxicologically relevant. Relative liver weight was slightly increased in males in the low-dose (5%), mid-dose (6%) and high-dose groups (8%). In high-dose females, a slight decrease in relative spleen weight (9%) was observed. As the observed effects were only slight (< 10%) and not accompanied by histopathological changes or effects on absolute weight, these slight weight changes were regarded as not toxicologically relevant. Histopathological findings in the lungs of five high-dose male animals included minimal alveolar histiocytosis compared with a slight alveolar histiocytosis in the lungs of only one control male animal. Considering that six control females as well as four high-dose females showed this finding, the effect was regarded as a spontaneous finding.

The NOAEL was 15 000 ppm (equal to 1033.5 mg/kg bw per day for males and 1161.4 mg/kg bw per day for females), the highest dose tested (Kaspers et al., 2008c).

The potential genotoxicity of M650F04 was investigated in three in vitro tests, with negative results. The studies are summarized in Table 11.

4. Observations in humans

All persons handling crop protection products are surveyed by regular medical examinations. There are no specific parameters available for effect monitoring of ametoctradin. Thus, the medical monitoring programme is designed as a general health checkup, with special interest in the primary target organs presumed to be relevant by analogy from animal experiments. The surveillance programme includes a general physical examination, including neurological status, red and white blood cell counts and liver enzymes. Adverse health effects suspected to be related to ametoctradin exposure have not been observed.

No human cases of intoxication or poisoning deriving from ametoctradin are known to BASF SE.

Neither data on exposure of the general public nor epidemiological studies are available for BASF SE, nor is BASF SE aware of any epidemiological studies performed by third parties.

Table 11. Results of genotoxicity studies with M650F04

End-point	Test system	Concentration/dose	Purity; batch	Result	Reference
In vitro					
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537; *E. coli* WP2*uvrA*; standard plate incorporation (1st experiment) and preincubation assay (2nd and 3rd experiments)	1st experiment: 20–5000 µg/plate (±S9) 2nd experiment: 20–5000 µg/plate (±S9) 3rd experiment: TA98: 2–500 µg/plate (±S9) TA100: 10–2500 µg/plate (±S9) TA1535: 20–5000 µg/plate (−S9) TA1535: 8–2000 µg/plate (+S9) TA1537: 2–500 µg/plate (+S9) *E. coli*: 0.4–100 µg/plate (+S9)	99.2%; L74-106	Negative	Schulz & Landsiedel (2007c)
Gene mutation (HPRT)	CHO-K1 cells	1st experiment: 131.3–2100 µg/ml (±S9) 2nd experiment: −S9: 500–2100 µg/ml +S9: 131.3–2100 µg/ml	99.2%; L74-106	Negative	Schulz & Hellwig (2006)
Chromosomal aberrations	V79 cells	1st experiment: 131.3–2100 µg/ml (±S9) (4 h exposure, 18 h sampling time) 2nd experiment: 131.3–2100 µg/ml (−S9) (18 h exposure, 18 h sampling time) 525.0–2100 µg/ml (−S9) (18 h exposure, 28 h sampling time) 131.2–2100 µg/ml (+S9) (4 h exposure, 28 h sampling time)	99.2%; L74-106	Negative	Schulz & Landsiedel (2007d)

S9, 9000 × *g* supernatant fraction of rat liver homogenate

Comments

Biochemical aspects

Following oral administration, ^{14}C-labelled ametoctradin underwent limited and saturable absorption from the gastrointestinal tract, but was quite widely distributed. Maximum plasma concentrations were observed within 1–2 hours after administration, and initial half-lives ranged from 2 to 3 hours. The AUC values indicate that internal exposure was not different in males and females. Excretion of ametoctradin occurred rapidly and independently of sex. Most of the administered dose (91–110%) was recovered within 168 hours after a single low or high dose and repeated high doses, with faeces as the main elimination route. Based on the amount of radioactivity excreted via bile and urine, the bioavailability of ametoctradin in rats was calculated to be about 40% of the administered dose at 50 mg/kg bw and about 20% of the applied dose at 500 mg/kg bw. The parent compound is metabolized by terminal oxidation of the octyl side-chain to the respective carboxylic acid (M650F09), with subsequent degradation of the carboxylic side-chain to give M650F06 and M650F01. In addition, conjugation of the respective oxidized side-chain with taurine and/or

glucuronic acid occurs, leading to metabolites M650F10 (taurine conjugate of M650F09), M650F11 (glucuronic acid conjugate of M650F06) and M650F12 (taurine conjugate of M650F06). Also, a minor metabolic step leads to the formation of M650F05 (ω-hetarylpentanoic acid). Several metabolites of ametoctradin were found in liver, kidneys, plasma and bile, with metabolite M650F06 being the most abundant.

Toxicological data

Ametoctradin has low acute oral and dermal toxicity (LD_{50} values > 2000 mg/kg bw) and low toxicity by inhalation (LC_{50} > 5.4 mg/l). No skin or eye irritation was observed after ametoctradin exposure. Ametoctradin was not a sensitizer in a Magnusson and Kligman maximization test or in the murine local lymph node assay.

In repeated-dose toxicity studies in mice, rats and dogs, no consistent toxicological findings were evident in any of the species at any dose tested up to the limit dose (around 1000 mg/kg bw per day) or after any study duration. Ametoctradin was extensively tested in a comprehensive set of current guideline studies, including short-term studies of toxicity, long-term studies of toxicity and carcinogenicity, studies of reproductive and developmental toxicity, neurotoxicity studies and an immunotoxicity study.

In the long-term studies of toxicity and carcinogenicity, no treatment-related changes in tumour incidence were observed.

The Meeting concluded that ametoctradin was not carcinogenic in mice or rats.

Ametoctradin was tested for genotoxicity in an adequate range of in vitro and in vivo studies. No evidence for genotoxicity was observed in any of these tests.

The Meeting concluded that ametoctradin was not genotoxic.

In view of the lack of genotoxicity and the absence of carcinogenicity in mice and rats, the Meeting concluded that ametoctradin is unlikely to pose a carcinogenic risk to humans.

The toxicity of several soil metabolites of ametoctradin was examined. Ninety-day dietary toxicity studies in rats were performed with metabolites M650F03 and M650F04, metabolites that were not found in the rat. In the 90-day dietary toxicity studies, no adverse effects were observed after exposure to either M650F03 or M650F04 up to the limit dose (i.e. 15 000 ppm, equivalent to about 1000 mg/kg bw per day). The genotoxic potential of three soil metabolites, M650F02, M650F03 and M650F04, was tested in several in vitro and in vivo studies. All were negative for genotoxicity.

The Meeting concluded that the existing database on ametoctradin was adequate to characterize the potential hazards to fetuses, infants and children.

Toxicological evaluation

From the animal studies with ametoctradin, no adverse effects were observed at or near the limit dose of approximately 1000 mg/kg bw per day. The Meeting concluded that it was not necessary to establish an acceptable daily intake (ADI) for ametoctradin. This was based on a reasonable estimate of a likely maximal intake of the residues of a pesticide arising from the daily diet. In the 2004 JMPR report (Annex 1, reference 101) and in more detail in the publication by Solecki et al. (2005) on guidance on setting acute reference doses (ARfDs), a maximum cut-off of 5 mg/kg bw for the ARfD was suggested, based on food consumption estimates and maximum residue levels in foods. This cut-off would equate to a NOAEL of 500 mg/kg bw per day in an animal study, with the application of the default uncertainty factor of 100. A similar principle was considered by the Meeting to be applicable in setting an extreme upper bound for the ADI, noting that the long-term daily dietary exposure for the residues of a particular pesticide will be less than the international estimate of short-term dietary intake (IESTI) for the residues of that pesticide. A cut-off for the ADI could be refined, taking into account long-term high-level consumption.

The Meeting concluded that it was not necessary to establish an ARfD for ametoctradin in view of the absence of acute toxicity or any other effect that could be elicited by a single dose.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen-month study of toxicity and carcinogenicity[a]	Toxicity	6000 ppm, equal to 1099 mg/kg bw per day[b]	—
		Carcinogenicity	6000 ppm, equal to 1099 mg/kg bw per day[b]	—
Rat	Two-year study of toxicity and carcinogenicity[a]	Toxicity	15 000–22 500 ppm, equal to 871 mg/kg bw per day[b]	—
		Carcinogenicity	15 000–22 500 ppm, equal to 871 mg/kg bw per day[b]	—
	Two-generation study of reproductive toxicity[a]	Parental toxicity	939 mg/kg bw per day[b]	—
		Offspring toxicity	939 mg/kg bw per day[b]	—
		Reproductive toxicity	939 mg/kg bw per day[b]	—
	Developmental toxicity study[c]	Maternal toxicity	1000 mg/kg bw per day[b]	—
		Embryo and fetal toxicity	1000 mg/kg bw per day[b]	—
	Acute neurotoxicity study[c]	Neurotoxicity	2000 mg/kg bw per day[b]	—
Rabbit	Developmental toxicity study[c]	Maternal toxicity	1000 mg/kg bw per day[b]	—
		Embryo and fetal toxicity	1000 mg/kg bw per day[b]	—
Dog	One-year study of toxicity[a]	Toxicity	30 000 ppm, equal to 848 mg/kg bw per day[b]	—

[a] Dietary administration.
[b] Highest dose tested.
[c] Gavage administration.

Estimate of acceptable daily intake for humans

Unnecessary

Estimate of acute reference dose

Unnecessary

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposure

Critical end-points for setting guidance values for exposure to ametoctradin

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Approximately 20% at high dose (500 mg/kg bw) and 40% at low dose (50 mg/kg bw)
Dermal absorption	No information on the pure active substance
Distribution	Widely distributed
Potential for accumulation	None
Rate and extent of excretion	Rapid and complete
Metabolism in animals	Limited; several metabolites, with M650F06 being most abundant
Toxicologically significant compounds in animals, plants and the environment	None
Acute toxicity	
Rat, LD_{50}, oral	> 2000 mg/kg bw per day
Rat, LD_{50}, dermal	> 2000 mg/kg bw per day
Rat, LC_{50}, inhalation	> 5.4 mg/l air (4 h, nose only)
Rabbit, dermal irritation	Non-irritant
Rabbit, ocular irritation	Non-irritant
Dermal sensitization	Not sensitizing (Magnusson & Kligman and local lymph node assay)
Short-term studies of toxicity	
Target/critical effect	No adverse effects at the limit dose
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	No adverse effects at the limit dose
Carcinogenicity	No carcinogenic potential
Genotoxicity	
	No genotoxic potential
Reproductive toxicity	
Reproduction target/critical effect	No adverse effects at the limit dose
Developmental toxicity	
Developmental target/critical effect	No adverse effects at the limit dose
Neurotoxicity	
Acute neurotoxicity	No neurotoxicity at the limit dose
Other toxicological studies	
Immunotoxicity studies	No immunotoxicity at the limit dose
Studies performed on metabolites or impurities	M650F02: Not genotoxic M650F03: Not genotoxic; no effects at the limit dose in 90-day rat study M650F04: Not genotoxic; no effects at the limit dose in 90-day rat study
Medical data	
	Limited information; new compound

Summary

	Value	Study	Safety factor
ADI	Unnecessary	—	—
ARfD	Unnecessary	—	—

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BENTAZONE

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Explanation

Bentazone (Figure 1) is the International Organization for Standardization (ISO)–approved common name for 3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide (International Union of Pure and Applied Chemistry), with Chemical Abstracts Service number 25057-89-0. Bentazone is a post-emergence herbicide used for selective control of broadleaf weeds and sedges in beans, rice, corn, peanuts, mint and others. It acts by interfering with photosynthesis.

Figure 1. Chemical structure of bentazone

Bentazone was first evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 1991, when an acceptable daily intake (ADI) of 0–0.1 mg/kg body weight (bw) was established on the basis of a no-observed-adverse-effect level (NOAEL) of 9 mg/kg bw per day (for increased clotting times and increased output of urine with decreased specific gravity) in a long-term study of toxicity in rats and using a safety factor of 100. In 1998, the Meeting re-evaluated bentazone and data on 6-hydroxybentazone, a metabolite of bentazone. The Meeting concluded that 6-hydroxybentazone was less toxic than bentazone and reaffirmed the ADI of 0–0.1 mg/kg bw. Because data were not evaluated to establish an acute reference dose (ARfD), the Meeting in 2004 re-evaluated bentazone and concluded that the establishment of an ARfD was not necessary.

Bentazone is being reviewed at the present meeting as part of the periodic re-evaluation programme of the Codex Committee on Pesticide Residues.

Since the 2004 JMPR review, no relevant new studies have been provided. Two published literature studies on the effects of bentazone on spermatogenesis in mice and on litter size and postnatal growth in rats were submitted. Most of the studies do not comply with good laboratory practice (GLP), as they were generated before implementation of GLP.

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

Mice

[^{14}C]Bentazone was administered intravenously in corn oil to C57 mice (number of animals not stated). Mice were housed in metabolism cages and provided with feed and water ad libitum. More than 95% of the administered radioactivity was recovered in the urine within 48 hours. Only traces (< 1%) were excreted in the faeces. Unchanged bentazone was the major constituent in urine; 76.83% ± 3.61% of the radioactivity recovered in the first 7 hours was identified as bentazone. The study was conducted before the implementation of GLP (Booth, 1974).

Rats

Single oral doses of 0.8 mg [^{14}C]bentazone (0.37 MBq per animal; labelled in the benzene ring) were administered by gavage in 50% aqueous ethanol to male and female Sprague-Dawley (CFY) rats (200–250 g). Urine was collected at 3, 6, 12 and 24 hours after dosing and daily thereafter. Faeces were collected daily. Expired gases from two animals were monitored for $^{14}CO_2$. Rats were sacrificed after 4 days. Young male rats were given total oral doses of 2.4 mg [^{14}C]bentazone, sacrificed at different intervals after dosing and subjected to whole-body autoradiography. Three male rats (150–175 g) with biliary cannulae were dosed as above, and bile was collected at hourly intervals for 40 hours; urine and faeces were also collected.

Oral doses of [^{14}C]bentazone were rapidly and almost quantitatively absorbed in rats. The radioactivity was quite rapidly excreted, mostly in the urine (approximately 91% within 24 hours), with only traces secreted into bile (0–2.9%). Over 4 days, less than 1% of the total dose was excreted in the faeces, and less than 0.02% in the expired air. Small amounts of radioactivity (0.54%) remained in the carcass after 4 days. Autoradiography showed that, after 1 hour, levels of radioactivity were high in the stomach, liver, heart and kidney, but undetectable in the brain or spinal cord. As determined by thin-layer chromatography, most of the radioactivity in the urine (84%) was unchanged bentazone. Two minor unidentified metabolites were detected, accounting for 2.3% and 0.8% of the radioactivity, respectively.

The study is scientifically valid, although it was conducted prior to the implementation of GLP (Hathway et al., 1971).

The bioavailability and metabolic fate of [^{14}C]bentazone free acid and [^{14}C]bentazone sodium salt were compared after oral administration to two groups of 12 adult Sprague-Dawley (CFY) rats (200–250 g). The first group received non-radioactive bentazone free acid (4 mg/kg bw dissolved in aqueous ethanol) once daily for 7 days. Twenty-four hours after the seventh dose, each rat received [^{14}C]bentazone free acid (4 mg/kg bw dissolved in aqueous ethanol), and blood samples were removed from a tail vein at intervals during 24 hours. The second group was treated in the same way, except bentazone sodium salt was used (dose equal to 4 mg/kg bw as the free acid).

For an excretion–retention study, three rats of each sex were orally intubated with [^{14}C]bentazone sodium (dose equal to 4 mg/kg bw as the free acid) in aqueous ethanol. Urine was collected from 0 to 6 hours and from 6 to 24 hours and then at 24-hour intervals over 5 days. Faeces were collected at 24-hour intervals for 48 hours and then as a single sample up to 5 days. After 5 days, the rats were killed.

After oral administration of either [^{14}C]bentazone free acid or the sodium salt to rats (4 mg/kg bw), no significant differences were detected in the maximal plasma concentrations of radioactivity or in the plasma half-lives of elimination. The mean time of occurrence of maximal concentrations of plasma radioactivity was shorter after dosing with the free acid (approximately 0.8 hour) than with the sodium salt (approximately 1.1 hours), although the difference was not statistically significant. Thus, the rate and extent of absorption were similar after administration of the sodium salt and free acid forms of bentazone.

An oral dose of [^{14}C]bentazone sodium salt (4 mg/kg bw) was well absorbed and rapidly eliminated; 6 hours after dosing, means of 63% (males) and 51% (females) of the dose had been excreted in the urine, and after 24 hours, means of 90% and 91% had been eliminated in males and females, respectively. Faecal excretion of radioactivity over 5 days accounted for 1.8% and 1.0% of the dose in males and females, respectively; no radioactivity was detected in the carcass at this time. Radioactivity excreted in the urine was mainly unchanged bentazone (85%). No significant differences were found in the pharmacokinetics or metabolism of bentazone sodium salt compared with bentazone free acid.

The study was conducted prior to the implementation of GLP, but is scientifically acceptable (Chasseaud et al., 1979).

To ascertain the extent of urinary excretion after oral dosing, a single oral dose of [U-phenyl-^{14}C]bentazone sodium salt (radiochemical purity > 97%) was administered as an aqueous solution to adult male CD rats (approximately 220 g) at a dose equal to 4 mg/kg bw as the free acid. Rats were sacrificed at different times (four per group) up to 72 hours after dosing. Radioactivity in the urine was checked during various intervals (0–6, 6–12 and 12–24 hours) post-administration. The metabolite pattern in combined urine samples collected during these intervals was investigated by thin-layer chromatography. Means of 65%, 15% and 3% of the dose were excreted in the urine as unchanged bentazone during 0–6 hours, 6–12 hours and 12–24 hours, respectively, in sum (83%) representing more than 90% of all radioactivity excreted in the urine. About 2% of the dose corresponded to 6-hydroxybentazone. Most of the remaining radioactivity (approximately 2% of the dose) was associated with polar material at the origin of the chromatogram. The reference compound 8-hydroxybentazone did not correspond to any of the radioactive components found in this experiment. These results indicate that orally administered bentazone is excreted in the urine almost entirely unchanged (83% of total dose), with traces (2%) of 6-hydroxybentazone and polar material.

The study was conducted according to the principles of GLP. A quality assurance (QA) statement was attached (Hawkins et al., 1986a).

The kinetics and metabolism of [^{14}C]bentazone were investigated in CD rats (nominal age 8 weeks). The radiochemical purity of [^{14}C]bentazone used was greater than 99%. In a preliminary experiment, two rats of each sex (205–210 g) were gavaged with a single oral high dose (198 mg/kg

bw) in order to determine whether significant amounts of ^{14}C were eliminated in the expired air. Urine, faeces and expired air were collected separately and radioassayed at 24-hour intervals for 5 days, at which time the animals were sacrificed for analysis of residual radioactivity in the carcass. By 120 hours post-dosing, less than 0.03% of the radioactivity had been eliminated in the expired air.

The details of the main experiment are shown in Table 1.

Table 1. Experimental design of a kinetics and metabolism study in rats

No.	Experiment	No. of animals	Mean body weight (g)	Mean dose (mg/kg bw)
1	Single oral low dose	5M + 5F	199 (191–209)[a]	3.8 (3.6–4.0)[a]
2	Single oral high dose	5M + 5F	200 (195–204)	205 (200–210)
3	Single oral low dose to rats pretreated for 14 days with an oral low dose of unlabelled bentazone	5M + 5F	215 (177–241)	3.6 (3.3–3.7) 4.0[b]
4	Single low intravenous dose of sodium salt	5M + 5F	202 (194–207)	4.1[c] (3.9–4.3)

From Hawkins et al. (1987)
F, female; M, male
[a] Range given in parentheses.
[b] Unlabelled bentazone.
[c] Expressed as bentazone free acid.

Urine was collected at 8 and 24 hours post-dosing and thereafter over 24-hour periods for a total of 120 hours. Faeces were collected at 24-hour intervals for up to 120 hours. The animals were sacrificed at the end of the collection period for determination of residual radioactivity in tissues. Blood and the following organs and tissues were sampled for radioactivity: liver, kidneys, thyroid, spleen, adrenals, heart, brain, lungs, pancreas, ovaries/testes, uterus, gastrointestinal tract and samples of muscle, bone marrow and fat.

In a biliary excretion study, three rats of each sex per group (average weight 200 g) were treated with a single oral low dose (3.6 mg/kg bw; range 3.3–3.9 mg/kg bw) or a single oral high dose (195 mg/kg bw; range 180–210 mg/kg bw) of bentazone. Bile was collected from bile duct cannulae at 1.5-hour intervals for up to 48 hours, at which time the animals were sacrificed to assess carcass radioactivity.

For a plasma level study, five rats of each sex per group were treated as shown in Table 2.

Table 2. Experimental design of a plasma level study in rats

No.	Type of experiment	No. of animals	Mean body weight (g)	Mean dose (mg/kg bw)
1	Single oral low dose	5M + 5F	203 (198–208)[a]	3.6 (3.6–3.7)[a]
2	Single oral high dose	5M + 5F	204 (197–208)	197 (190–200)
3	Single oral low dose (sodium salt)	5M + 5F	203 (194–209)	4.1[b] (3.9–4.2)
4	Single low intravenous dose (sodium salt)	5M + 5F	200 (194–204)	4.5[b] (4.3–4.7)

From Hawkins et al. (1987)
F, female; M, male
[a] Range given in parentheses.
[b] Expressed as bentazone free acid.

Blood samples were taken from the tail and vein at 0.25, 0.5, 1, 2, 3, 4, 6 and 24 hours post-dosing and at 24-hour intervals until plasma radioactivity had declined to the limit of detection. In addition, one sample was collected at 5 minutes from rats dosed intravenously with bentazone sodium.

For a tissue distribution study, 10 male and 5 female rats were given low oral doses (4 mg/kg bw) of [^{14}C]bentazone once daily for 7 days, and one rat of each sex was sacrificed at various time points (0.5, 6, 24, 72 and 120 hours after the last dose) for assay of radioactivity in the following organs and tissues: liver, kidneys, heart, lungs, brain, eyes, gonads (testes or ovaries), spleen, pancreas, adrenals, thyroid, gastrointestinal tract, uterus and samples of muscle, bone marrow and fat. The remaining carcass was discarded. In addition, males were also sacrificed at various time points (0.5, 6, 24, 72 and 120 hours after the last dose) for whole-body autoradiography.

Metabolite characterization studies were performed with representative urine and faecal samples collected during the first 24 hours post-dosing. Structural characterization (gas chromatography/mass spectrometry) of the major urinary radioactive component was performed. Quantification of metabolites in raw urine from animals of the various dose groups of the main study was done by high-performance liquid chromatography. Additionally, representative urine samples were treated with β-glucuronidase/sulfatase to assess the extent of conjugation of bentazone metabolites.

Single intravenous low dose: Five days after administration of a single intravenous dose of [phenyl-U-^{14}C]bentazone sodium salt (mean dose equal to 4.1 mg/kg bw as the free acid) to rats, approximately 95.4% (males) and 90.2% (females) of the dose were accounted for. Urine accounted for at least 93.87% of the dose in males and 88.96% in females. Most (91.5% and 85.8% in males and females, respectively) of the elimination in urine was complete within the first 24 hours. Faecal elimination accounted for about 1.18% (males) and 0.51% (females) of the dose.

Total radioactive residue in the carcass amounted to 0.32% of the dose in females; no data were available for males. Radioactive residues in kidneys amounted to 0.019 µg/g and 0.026 µg/g in males and females, respectively; and in the uterus, to 0.002 µg/g. Residues in other tissues were at or below the limit of measurement (twice the background radioactivity).

Single low oral dose: Five days after administration of a single oral dose of [phenyl-U-^{14}C]bentazone free acid (mean 3.8 mg/kg bw) to rats, approximately 91.95% (males) and 90.05% (females) of the dose were accounted for. Urine accounted for at least 89.8% (males) and 88.13% (females) of the dose; most of the elimination in urine (86.7% and 83.7% in males and females, respectively) was complete within the first 24 hours. Faecal elimination accounted for about 1.5% of the dose in males and 0.76% of the dose in females. Radioactive residue in the carcass amounted to 0.48% (males) and 0.69% (females) of the dose. Residues in tissues were at or below the limit of measurement (twice the background radioactivity).

Single high oral dose: Five days after administration of a single oral dose of [phenyl-U-^{14}C]bentazone free acid (mean 205 mg/kg bw) to rats, approximately 97.13% (males) and 95.78% (females) of the dose were accounted for. Urine accounted for at least 94.32% (males) and 93.03% (females) of the dose. Most of this elimination in urine (92.0% and 91.0% of the dose in males and females, respectively) was complete within the first 24 hours. Faecal elimination accounted for about 2.27% (males) and 2.00% (females) of the dose. Total radioactive residue in the carcass amounted to 0.24% and 0.17% of the dose in males and females, respectively. Residues in tissues were at or below the limit of measurement (twice the background radioactivity).

Single low oral dose with pre-dosing: One hundred and twenty hours after administration of a single oral dose of [phenyl-U-^{14}C]bentazone free acid (mean 3.6 mg/kg bw) to rats, preceded by single daily oral doses of non-radioactive bentazone free acid (4 mg/kg bw) for 14 days, approximately 96.81% and 92.5% of the dose were accounted for in males and females, respectively. Elimination in the urine accounted for at least 95.86% of the dose in males and 90.49% in females. Most (94.1% and 85.2% of the dose in males and females, respectively) of the elimination in urine was complete within the first 24 hours. Elimination in the faeces accounted for about 0.92% of the

dose in males and 1.44% of the dose in females. Total radioactive residue in the carcass amounted to 0.5% of the dose in females and was undetectable in males. Residues in tissues were at or below the limit of measurement (twice the background radioactivity).

Biliary excretion studies: Excretion of radioactivity in bile was very limited using bile duct–cannulated rats. At the high dose, biliary excretion amounted to 0.80% (females) and 1.84% (males) of the total dose. At the low dose, means of 0.2% (females) and 1.3% (males) of the radioactivity administered were excreted in the bile. Biliary excretion was essentially complete by 24 hours.

Time course of plasma radioactivity: Radioactivity in plasma reached a maximum by 15 minutes at the low oral dose (free acid or sodium salt) and by 1 hour at the high dose. Determination of the area under the plasma concentration–time curve (AUC) per unit dose revealed significantly higher values for the high-dose groups compared with the low-dose groups (Table 3). AUC values for females dosed with low oral doses were significantly lower (nearly half) than AUC values in females dosed intravenously; the corresponding values for males were not significantly different.

Table 3. Mean areas under the plasma concentration–time curves in rats dosed with [phenyl-U-^{14}C]bentazone (free acid or sodium salt)

Test group (dose in males/females, in mg/kg bw)	Form of bentazone	AUC (µg·h/ml) / dose (mg/kg bw)	
		Males	Females
Oral low (3.7/3.6)	Free acid	8.0 ± 2.6	3.5 ± 0.7
Oral high (196/198)	Free acid	15.0 ± 3.8	11.0 ± 3.9
Oral low (4.1/4.0)	Sodium salt	6.2 ± 1.8	3.5 ± 1.1
Intravenous low (4.5/4.4)	Sodium salt	6.0 ± 2.0	6.8 ± 1.2

From Hawkins et al. (1987)

Time course of tissue levels of radioactivity: Concentrations of radioactivity were highest at 0.5 hour after dosing in tissues obtained from pairs of rats sacrificed at different times after the last of seven low-level oral doses of [^{14}C]bentazone. At this time, concentrations in most tissues ranged from 0.1 to 5 µg/g. Higher concentrations were confined to the gastrointestinal tract, kidney, thyroid and plasma (5–20 µg/g). At 6 hours, concentrations of radioactivity were generally in the region of 0.05–1 µg/g, apart from higher concentrations in the gastrointestinal tract, kidney, thyroid and plasma (0.5–5 µg/g). At 24 and 120 hours after the last dose, concentrations of radioactivity in all tissues examined were below 0.1 µg/g, except in the thyroid (< 0.3 µg/g).

A comparison of the tissue distribution after a single and seven daily doses of [^{14}C]bentazone revealed no evidence of radioactivity accumulation after repeated dosing. Whole-body autoradiography in general confirmed the distribution reported above. Some affinity of the compound to the keratinized layer of squamous epithelium of the non-fundic mucosa of the stomach was detected, as moderate levels of radioactivity were present there. In all tissues, there was a steady disappearance of label with time.

Metabolite characterization studies: Rat urine samples collected over a 24-hour period were analysed by high-performance liquid chromatography. Parent bentazone amounted to 80.63–91.02% of the dose in males and 77.37–88.95% of the dose in females. 6-Hydroxybentazone was present at up to 6.34% of the dose. The isomeric 8-hydroxybentazone was present in trace amounts (0.0–0.23% of the dose). Although the amounts of bentazone excreted in the urine appear to be slightly lower in the intravenously dosed rats, there were no major dose-dependent or pretreatment-dependent differences among groups. The level of glucuronide or sulfate conjugation was negligible or non-existent.

Characterization of metabolites in tissues was done in liver and kidney of rats sacrificed 6 hours after the last of seven daily oral doses of [phenyl-U-^{14}C]bentazone. The only compound detected was parent bentazone. The hydroxylated metabolites were not seen in tissue extracts,

presumably because the method was not sufficiently sensitive to detect metabolites that accounted for less than about 10% of the dose. Metabolites in bile and faeces were not characterized.

The results of these rat studies indicate a rapid absorption, distribution and primarily renal excretion of [^{14}C]bentazone, with no appreciable differences for the various dosing regimens applied. Biliary excretion was minimal. There was no significant difference in the pharmacokinetics of orally administered bentazone free acid and its sodium salt. However, some sex-related differences were observed with regard to AUC values for radioactivity. For females administered low doses of bentazone sodium, AUC values were significantly lower after oral administration (nearly half) than AUC values in females dosed intravenously; corresponding values for males were not significantly different. There was no evidence for accumulation of bentazone or its metabolites in tissues.

An investigation of urinary metabolites after the different dosing regimens indicated that bentazone was excreted in urine mostly unchanged (77–91% of the dose). The minor urinary metabolite was 6-hydroxybentazone, accounting for 1–6% of the dose, and was clearly distinguished from 8-hydroxybentazone, which was found only in male rats in trace amounts (ranging from 0.16% to 0.23% of the total dose). In tissues, only parent bentazone was detected using the available analytical methodology.

These studies were conducted in compliance with GLP, and a QA statement was supplied (Hawkins et al., 1987).

Rabbits

Three New Zealand albino male rabbits were administered a single oral dose of bentazone (^{14}C-labelled in the phenyl ring) at a dose of 5 mg/kg bw. Four animals were used as controls. The animals had free access to feed and drinking-water. They were housed in metabolism cages for faeces and carbon dioxide collection. Urine was collected daily via catheter. Blood was collected prior to and at 1, 2, 3, 4.5, 5.5, 9, 11, 13.5, 20 and 24 hours after dosing. The rabbits were sacrificed 6 days after treatment.

Total recovery of radioactivity averaged 93.5%, with 90.3% excreted in the first 24 hours. Most of the radioactivity was excreted in the urine (89.7%), and the remainder in the faeces (3.8%). Less than 0.1% was in the expired air. Tissue levels were below 0.02 mg/kg.

Blood levels reached a peak 2.5 hours after dosing. The elimination half-life of radioactivity in the blood was 2 hours and 12 minutes. Analytical investigation revealed that less than 1% of the radioactivity recovered could be assigned to 6-hydroxybentazone and 8-hydroxybentazone. The hydroxy compounds were eliminated in their free form in the urine, and not as conjugates with glucuronic acid.

The results after a single oral administration of [^{14}C]bentazone at 5 mg/kg bw to male rabbits were substantially similar to those obtained with rats. About 90% of the radioactivity administered was found in the urine, and nearly 4% in the faeces, within 24 hours. Six days after dosing, tissue residues were very low.

This study was performed prior to the implementation of GLP (Otto, 1974).

1.2 Metabolism

The metabolism of bentazone was investigated in a number of toxicokinetic studies following oral (rat and rabbit) or intravenous administration (mouse); these studies are described above. Bentazone was only poorly metabolized, with the parent compound being the predominant excretion product. Only small amounts of 6-hydroxybentazone and 8-hydroxybentazone could be detected. In rats, rabbits and mice, no conjugated products were found.

The metabolic pathway of bentazone is illustrated in Figure 2.

Figure 2. Metabolic pathway of bentazone

bentazone

6-OH-bentazone

8-OH-bentazone

2. Toxicological studies

2.1 Acute toxicity

Acute oral toxicity studies are available for a number of species (i.e. rat, guinea-pig, cat, dog and rabbit). Acute oral median lethal doses (LD_{50}s) are between 850 and 2470 mg/kg bw. No significant differences in LD_{50} were found between the free acid and the sodium salt. Signs of toxicity included dyspnoea, apathy, cachexia, staggering and poor general state. The acute dermal toxicity LD_{50} in rat was more than 5000 mg/kg bw. The 8-hour exposure inhalation toxicity (median lethal concentration, or LC_{50}) in rat was greater than 1.2 mg/l, whereas the 4-hour exposure LC_{50} was greater than 5.1 mg/l (mass median aerodynamic diameter [MMAD] 6.4 µm); no signs of toxicity were observed at this dose. Bentazone is not a skin irritant but was a moderate eye irritant in rabbits. It is a skin sensitizer in guinea-pigs. Intraperitoneal LD_{50}s were between 316 and 975 mg/kg bw in rats and mice. Results are summarized in Table 4.

All these studies were conducted prior to the implementation of GLP.

2.2 Short-term studies of toxicity

(a) Oral administration

Short-term studies of oral toxicity in mice, rats and dogs were conducted.

Mice

In a 30-day dietary study, female B6C3F1/CRJ mice (28 days old; six of each sex per dose) were given diets containing bentazone (purity 93.9%) at a concentration of 0, 400, 2000, 5000 or 10 000 parts per million (ppm) (equal to 0, 90, 407, 905 and 1469 mg/kg bw per day for males and 0, 100, 487, 1004 and 1663 mg/kg bw per day for females, respectively). The mice were observed daily and weighed twice per week. Feed consumption and water consumption were measured twice per week. After 30 days, the animals were killed and subjected to necropsy, and selected organs were weighed and processed for histopathology. Blood was taken from some mice (three of six controls of each sex, three of six male mice at 2000 ppm and two of six female mice at 2000 and 5000 ppm) for measurement of clinical pathology parameters.

Table 4. Summary of acute toxicity studies with bentazone

Species	Strain	Sex	Route	Form, batch no. and purity (if provided)	LD_{50} (mg/kg bw) / LC_{50} (mg/l)	Result	Reference
Rat	Sprague-Dawley	M + F	Oral	Acid	~850	[a]	Zeller & Hofmann (1969)
Rat	Sprague-Dawley	M + F	Oral	Acid	1050	[b]	Hofmann (1972a)
Rat	Sprague-Dawley	M + F	Oral	Acid	1220	[c]	Hofmann (1973a)
Rat	Sprague-Dawley	M + F	Oral	Sodium salt	1480 (equal to 1356 as free acid)	[d]	Hofmann (1973b)
Rat	Sprague-Dawley (CRJ)	M + F	Oral	Acid, lot no. 270778, 94.6%	M: 2340 F: 2470	[e]	Toyoshima (1978)
Rat	Wistar	M + F	Oral	NA	1710	[f]	Kirsch & Hildebrand (1983b)
Rat	Wistar	M + F	Oral	Free acid, batch N 169, 93.9%	M: 1780 F: 1470 Combined: 1640	[g]	Kirsch & Hildebrand (1983a)
Guinea-pig	NA	M + F	Oral	Sodium salt	1100 (equal to 1000 as free acid)	[h]	Hofmann (1974)
Guinea-pig	NA	M + F	Oral	Free acid	1100	[i]	Hofmann (1991)
Rabbit	NA	M + F	Oral	—	750	[j]	Zeller & Birnstiel (1969)
Rabbit	New Zealand White	M + F	Oral	—	1139	[k]	Neuschl & Kacmar (1993)
Cat	NA	M + F	Oral	—	~500	[l]	Zeller & Magoley (1970a)
Dog	Beagle	M + F	Oral	—	—	[m]	Zeller & Magoley (1970b)
Rat	Sprague-Dawley	M + F	Dermal	—	> 2500	No toxic signs	Zeller (1969)
Rat	CRJ:SD	M + F	Dermal	Acid, batch 270778, 94.6%	> 5000	No toxic signs	Toyoshima (1978)

Table 4 (continued)

Species	Strain	Sex	Route	Form, batch no. and purity (if provided)	LD_{50} (mg/kg bw) / LC_{50} (mg/l)	Result	Reference
Rat	Sprague-Dawley	M + F	Inhalation (8 h)	—	> 1.2	[n]	Hofmann & Zeller (1969a)
Rat	SPF Wistar/ Chbb:THOM	M + F	Inhalation (dust aerosol) (4 h)	Acid, batch N 187, 97.8%	> 5.1 (MMAD 6.4 µm)	No significant toxicity	Klimisch (1986)
Rabbit	White Vienna	M + F	Skin irritation	Lot 83/5, 50% aqueous formulation	—	No skin irritation potential	Kirsch & Hildebrand (1983c)
Rabbit	White Vienna	M + F	Eye irritation	Acid, lot 83/5	—	Shows an eye irritation potential	Kirsch & Hildebrand (1983d)
Guinea-pig	Pirbright White, Dunkin-Hartley, HOE DMPK	F	Skin sensitization	Acid, batch MS 2 F 22, 94%	—	Has skin sensitizing properties	Kieczka & Kirsch (1986)
Guinea-pig	Pirbright White, Dunkin-Hartley, HOE DHPK (SPF-LAC)	F	OET for sensitizing potential	Lot WH 4976, 60%	—	[o]	Klecak (1977); Kieczka (1986); Kieczka & Hildebrand (1986)
Mouse	NMRI	M + F	Intraperitoneal	—	~400	[p]	Hofmann & Zeller (1969b)
Mouse	CRJ:ICR	M + F	Intraperitoneal	270778, 94.6%	M: 494 F: 505	[q]	Toyoshima et al. (1978b)
Mouse	CRJ:ICR	M + F	Subcutaneous	270778, 94.6%	M: 655 F: 580	[r]	Toyoshima et al. (1978b)
Rat	Sprague-Dawley	M + F	Intraperitoneal	—	344	—	Hofmann (1972b)
Rat	Sprague-Dawley (CRJ)	M + F	Intraperitoneal	270778, 94.6%	M: 403 F: 407	[s]	Toyoshima et al. (1978a)
Rat	Sprague-Dawley (CRJ)	M + F	Subcutaneous	270778, 94.6%	M: 970 F: 975	[t]	Toyoshima et al. (1978a)
Rat	Wistar	M + F	Intraperitoneal	—	> 316 < 383	[u]	Kirsch & Hildebrand (1983e)

F, female; M, male; NA, not available; OET, open epicutaneous test

[a] Signs of toxicity in the 200–1600 mg/kg bw dose groups included dyspnoea, apathy and piloerection. No abnormality was detected on days 3–5 in surviving animals.

[b] Signs of toxicity in the 1250–2000 mg/kg bw dose groups included dyspnoea and apathy. The animals of the other dose groups did not show any signs of toxicity. Necropsy findings in animals that died were cardiac dilatation and congestive hyperaemia.

[c] Signs of toxicity noted at 1000 mg/kg bw and above were dyspnoea and red incrustations in eyes. Necropsy findings of the animals that died intercurrently were acute congestive hyperaemia, acute cardiac dilatation (right chamber) and liver putty coloured with lobular pattern. No abnormalities were noted at necropsy of animals sacrificed at the end of the study. No abnormality was detected from day 7 in the 1000–1600 mg/kg bw dose groups.

[d] Clinical signs observed were dyspnoea and prostration. Necropsy findings in animals that died were congestive hyperaemia and effects on heart, stomach, intestine and liver. No abnormalities were noted at necropsy of animals sacrificed at the end of the study. No abnormality was detected in surviving animals on days 2–3. Necropsy of animals that died showed bloody gastric ulceration and haemorrhagic contents in intestine.

[e] Signs of toxicity noted in all dose groups included decreased spontaneous motility, ventral position, clonic convulsions and abdominal respiration. Necropsy did not reveal abnormalities in either animals that died or animals sacrificed at the end of the study.

[f] Signs of toxicity noted in the 825–2610 mg/kg bw dose groups were dyspnoea, apathy, cachexia, staggering and poor general state. No signs of toxicity were seen in females of the 1780 mg/kg bw dose group and all animals of the 562 mg/kg bw dose group. The expected body weight gain was observed in the course of the study. Necropsy findings in animals that died were general congestion, spot-like hyperaemia and slight emphysema in lungs, ulcers and haemorrhages in the gastrointestinal tract, and anaemic colour and slight acinar pattern of the liver. Kidneys of one male animal of the highest dose group were sand coloured, and adrenals were loam coloured. No abnormalities were noted at necropsy of animals sacrificed at the end of the study. Necropsy of animals that died showed bloody gastric ulceration and haemorrhagic contents in intestine.

[g] Clinical signs observed were dyspnoea, apathy, staggering, opisthotonus, cachexia and poor general state. Body weight development was unaffected. Necropsy of animals that died showed bloody gastric ulceration and haemorrhagic contents in intestine.

[h] Signs of toxicity noted in the 1250 and 1600 mg/kg bw groups were prostration, apathy and tachypnoea. The animals of the other dose groups did not show any symptoms.

[i] Signs of toxicity noted in the 1200, 1600 and 3200 mg/kg bw groups were abdominal lateral position, apathy, tachypnoea, atonia and dyspnoea. The animals of the other dose groups did not show any symptoms. Necropsy findings of animals that died were acute congestion, acute cardiac dilatation and acute inflation of the lung. No abnormalities were noted at necropsy of animals sacrificed at the end of the study.

[j] Signs of toxicity noted in the 100, 500 and 2000 mg/kg bw dose groups were slight giddiness, anorexia and diarrhoea. This study was not acceptable.

[k] This was a summary of published literature. For male and female adult New Zealand White rabbits, a combined LD_{50} of 1139 mg/kg bw was calculated, with respiratory, cardiac and central nervous system symptoms occurring.

[l] Signs of toxicity noted in the 500–2000 mg/kg bw dose groups comprised titubation, vomiting, transient mydriasis, dysbasia, tremors, prostration, loss of rising reflex, atony, convulsions, opisthotonus, tetanic spasm and spastic paresis. Additionally, a slight body weight loss was noted at all dose levels. Necropsy findings of animals that died were foci of fatty degeneration and necrobiosis on the cut surface of the liver. No abnormalities were noted at necropsy of animals sacrificed at the end of the study.

[m] As a result of the vomiting of the animals in the higher dose groups, it was not possible to determine the acute oral LD_{50}.

[n] The results were not used for the evaluation.

[o] A sensitizing potential that could be of significance under conditions in practice can be assumed for application concentrations higher than 10% bentazone sodium.

[p] Signs of toxicity noted in the 200–800 mg/kg bw dose groups included dyspnoea, apathy, pronation and tremors. Female mice appeared to be more sensitive than males. Necropsy findings of animals that died were intra-abdominal adhesions. No abnormalities were noted at necropsy of animals sacrificed at the end of the study.

[q] Signs of toxicity noted in all dose groups comprised decreased spontaneous motility, ventral position, clonic convulsions and abdominal respiration. Necropsy did not reveal abnormalities in organs of either animals that died or animals sacrificed at the end of the study. In animals that died, traces of unabsorbed test compound were found at the injection sites.

[r] Signs of toxicity noted in all dose groups included decreased spontaneous motility, decreased response to external stimulus, such as sound and light, ventral position, clonic convulsions and abdominal respiration. Necropsy did not reveal abnormalities in organs of either animals that died or animals sacrificed at the end of the study. In animals that died, traces of unabsorbed test compound were found at the injection sites.

Table 4 (continued)

[s] Signs of toxicity noted in all dose groups were decreased spontaneous motility, ventral position, clonic convulsions and abdominal respiration. Necropsy did not reveal abnormalities in organs of either animals that died or animals sacrificed at the end of the study. In animals that died, traces of unabsorbed test compound were found at the injection sites.

[t] Signs of toxicity noted in all dose groups included decreased spontaneous motility, ventral position, clonic convulsions and abdominal respiration. Necropsy did not reveal any abnormalities in organs of either animals that died or animals sacrificed at the end of the study. In dead animals, traces of unabsorbed test compound were found at the injection sites.

[u] Signs of toxicity noted in the 316–562 mg/kg bw groups were dyspnoea, apathy, staggering, excitation, twitching and poor general state. Animals of the 261 mg/kg bw dose group did not show any effects. The surviving animals gained weight during the observation period.

At 10 000 ppm, all mice died within 30 days, whereas at 5000 ppm, six males and four females died. At 5000 and 10 000 ppm, the mice showed depression, skin pallor and low skin temperature. At 5000 and 10 000 ppm, decreases in body weight gain, feed consumption and water consumption were seen in both sexes. At necropsy of the dead animals at 5000 and 10 000 ppm, haemorrhages were seen in subcutaneous tissue, pia, lungs, thoracic, pericardial and abdominal cavities, thymus, orbits and skeletal muscles. Prothrombin time and partial thromboplastin time were prolonged at 2000 ppm in both sexes and at 5000 ppm in females. However, no such investigation was done at 400 ppm. On histopathological examination of animals receiving bentazone at 5000 and 10 000 ppm, there were haemosiderosis and extramedullary haematopoiesis in the spleen, haemorrhage and haemosiderosis in cardiac muscle, and haemorrhages in the cerebral cortex and pia.

Repeated administration of bentazone to mice led to an impairment of blood coagulation at a dose of 2000 ppm and above. The clinical signs and the haemorrhages observed at 5000 and 10 000 ppm are attributable to this effect. As the blood coagulation parameters were not investigated at 400 ppm and as there were effects at the next higher dose level of 2000 ppm, the NOAEL cannot be set at 400 ppm. The lowest-observed-adverse-effect level (LOAEL) is suggested to be 2000 ppm (equal to 407 and 487 mg/kg bw per day for males and females, respectively) on the basis of prolonged prothrombin and partial thromboplastin times at this dose. The study was not done according to GLP, and no QA statement was attached (Anonymous, 1981; Takehara & Tajima, 1982).

Rats

In a range-finding study, bentazone (purity 93.9%) was administered to Fischer 344 rats (34 days old; eight of each sex per dose) via the diet at a concentration of 0, 600, 1800, 5000 or 10 000 ppm (equal to 0, 64, 196, 554 and 1068 mg/kg bw per day for males and 0, 71, 217, 607 and 1132 mg/kg bw per day for females, respectively) for 31–33 days. Clinical observations were made twice a day. Body weight, feed consumption and water consumption were recorded twice a week. Clinical, haematological, gross pathological and histopathological examinations were carried out at the end of the study. Clinical chemistry examinations were not included. Organ weights of spleen, heart, pituitary, adrenal glands and ovaries were determined.

At 10 000 ppm, rats showed cyanosis of the skin of distal parts of the body and fading of the pigment of the fundus of the eyeball. One male rat died on day 10. Body weight gain of male rats of the 10 000 ppm dose group was significantly suppressed. A temporary decrease in feed consumption was observed in female animals, and water consumption was slightly decreased in both sexes. No clinical signs of toxicity were observed in animals of the other dose groups. Haematological examinations revealed a decrease in haemoglobin and haematocrit in male rats of the 10 000 ppm dose group and a fall in the mean red blood cell haemoglobin, whereas white blood cell count was significantly increased in this male group. The prothrombin and partial thromboplastin times were significantly prolonged in both sexes at this dose (Table 5). Findings of the one male animal that died comprised subcutaneous bleeding and bleeding from the thorax and thymus gland. Necropsy of animals sacrificed at the end of the study showed bleeding in various tissues and organs in seven

males and three females at 10 000 ppm. Absolute weights of heart and testicles were significantly decreased in male rats of the 10 000 ppm dose group, whereas the weights of liver and left kidney were significantly increased in females of this group. Histopathological examinations revealed bleeding from the renal cortex in one male rat and from the ovaries of two female rats at 5000 ppm. In the two lower dose groups, no substance-related findings were observed.

Table 5. Selected haematological findings (group means) in rats after administration of bentazone for 4 weeks

	Males					Females				
	0 ppm (*n* = 7)	600 ppm (*n* = 2)	1800 ppm (*n* = 2)	5000 ppm (*n* = 6)	10 000 ppm (*n* = 7)	0 ppm (*n* = 7)	600 ppm (*n* = 2)	1800 ppm (*n* = 2)	5000 ppm (*n* = 5)	10 000 ppm (*n* = 8)
Thromboplastin time (s)	17.1	17.3	19.3	22.7	27.2	14.4	14.2	15.3	15.4	15.4
(% change relative to control)	—	—	—	—	(59.1)	—	—	—	—	(6.9)
Partial thromboplastin time (s)	34	28.4	33.6	44.7	153.4	29.7	26.7	30.8	25.6	47.8
(% change relative to control)	—	—	—	(31.5)	(351)	—	—	—	—	(60.9)

From Itabashi et al. (1981)

Bentazone led to an impairment of blood coagulation in rats, and bleeding observed in several organs and anaemia were assessed to be related to this. The decreased absolute weights of heart and testes were attributed to the impaired body weight gain, rather than to compound administration itself. Liver and kidney weights were increased, although there were no related histopathological findings.

Under the conditions of this study, the NOAEL was 1800 ppm (equal to 196 mg/kg bw per day for males and 217 mg/kg bw per day for females), based on toxicity apparent at the top dose and equivocal findings, such as bleeding from the urogenital system, in some animals at 5000 ppm. The study was performed prior to implementation of specific test guidelines and was not GLP compliant (Itabashi et al., 1981).

In a 90-day dietary study, bentazone (batch and purity not given) was administered to Sprague-Dawley rats at 0, 70, 200, 800 or 1600 ppm in the diet (equivalent to 0, 3.5, 10, 40 and 80 mg/kg bw per day, respectively). At the start of the trial, the average weights of male and female rats were 124 and 118 g, respectively. Each dose group had 20 animals of each sex, with a further 10 of each sex at 0, 70 and 1600 ppm kept under observation for a post-trial period of 42 days without test substance administration. Clinical signs and feed consumption were checked daily, and body weight was determined weekly. In all animals, haematological and biochemical examinations as well as urine analysis were carried out. All animals were assessed gross pathologically and subjected to a histopathological examination.

No clinical signs of toxicity were observed. Feed consumption and body weight gain of all treated male rats were comparable with those of the control. At 1600 ppm, body weight gain of the female rats was slightly retarded. There were no differences in the absolute body weights of males and females in treated and control groups. The body weights of male and female rats in the highest dose group were slightly lower than those of the other groups. No treatment-related changes could be observed in haematological and biochemical examinations in test or control animals. There were no appreciable differences in the mean absolute weights of liver, kidneys or heart. The relative kidney

weight of male rats of the 1600 ppm group and of female rats of the two highest dose groups was increased when compared with control values. The relative liver weights of all treated rats did not differ from those of the controls. At 1600 ppm, male rats showed increased relative heart weights, and the liver to heart and kidney to heart weight ratios were higher than those of the controls. Female rats of the 70 ppm group exhibited lower relative heart weights, and the liver to heart weight ratio was increased at this dose level. Females of the 70, 800 and 1600 ppm groups exhibited increased kidney to heart weight ratios. In the withdrawal trial, these increased ratios proved to be reversible. Feed consumption of both sexes and body weight gain of the male animals remained unaffected during the post-observation period. Females of the 1600 ppm group exhibited lower body weight gains. No test substance–related macroscopic changes were found at necropsy of the test animals. Two animals of each of the 200 ppm and 1600 ppm groups were found to have detectable degeneration of the testicular tissue. No further histopathological changes occurred in any of the other organs. Although no histopathological changes were observed, the liver weight changes were assessed as indicative of a slight liver adaptation process induced by the administration of the test substance. The minor and inconclusive effects on organ weights noted at 70 and 200 ppm in single animals were assessed as incidental because there was a lack of a dose–response relationship.

In view of the above, the NOAEL was 200 ppm (equal to 10 mg/kg bw per day), based on increased relative kidney weight in females at 800 ppm (equal to 40 mg/kg bw per day) and slight effects on body weight gain at the top dose level. The study was conducted prior to implementation of any specific test guidelines or of GLP (Zeller & Kirsch, 1970).

Bentazone (ZNT No. 86/48; batch N 187; purity 97.8%) was administered to rats (Wistar KFM-Han) at a dietary concentration of 0, 400, 1200 or 3600 ppm (equal to 0, 25.3, 77.8 and 243.3 mg/kg bw per day for males and 0, 28.9, 86.1 and 258.3 mg/kg bw per day for females, respectively) in a 13-week oral toxicity study. The study comprised four groups, each containing 10 male and 10 female rats about 8 weeks of age and weighing 168–206 g (males) and 150–177 g (females). The reversibility of treatment-related changes was studied using 10 additional animals of each sex at dietary concentrations of 0 and 3600 ppm over a 4-week recovery period.

No signs of toxicity were noted. There were a total of three deaths at the highest dose. Two rats were found dead in their cages during the 9th and 12th weeks of treatment. Another high-dose female rat died during anaesthesia on the day of scheduled necropsy. There was no compound-related effect on feed consumption in any group. In the high-dose group, body weight gains were slightly reduced for females, leading to a 6% decrement (significant at 5% level) in mean terminal body weight relative to female controls after 13 weeks of treatment. Body weight gains noted for males of this dose group and for both sexes of the low- and mid-dose groups were similar to those of the respective control animals. Body weight gains of animals of the high-dose recovery group during the 4-week regression period were also similar to those of the respective control animals. No compound-related effect was noted in ophthalmoscopy. Haematological examinations revealed prolonged thromboplastin and partial thromboplastin times for male animals of the high-dose group. The prolonged coagulation times may reflect an inhibitory effect on blood clotting factors. This effect was found to be reversible at the end of the recovery period. The biological meaning of a shortened prothrombin time as seen in females is equivocal (Table 6).

There was increased total cholesterol in high-dose females, as well as an increased albumin fraction and albumin to globulin ratio for mid- and high-dose males. These changes were reversible (Table 7).

Table 6. Selected haematological findings (group means) in rats administered bentazone technical for 91 days

	Males				Females			
	0 ppm	400 ppm	1200 ppm	3600 ppm	0 ppm	400 ppm	1200 ppm	3600 ppm
Thromboplastin time (s)								
- at end of treatment period	13.5	13.2	13.2	15.8*	13.2	12.5*	12.4*	12.4*
- at end of recovery period	13.3	—	—	13.4	12.9	—	—	13.0
Partial thromboplastin time (s)								
- at end of treatment period	22.5	22.3	23.9	30.2*	20.4	21.3	20.8	21.8
- at end of recovery period	21.2	—	—	21.4	19.2	—	—	18.9

From Tennekes et al. (1987)
* $P \leq 0.05$ (Dunnett)

Table 7. Selected clinical chemistry findings (group means) in rats administered bentazone technical for 91 days

	Males				Females			
	0 ppm	400 ppm	1200 ppm	3600 ppm	0 ppm	400 ppm	1200 ppm	3600 ppm
Total cholesterol (mmol/l)								
- at end of treatment period	2.36	2.49	2.32	2.49	2.30	2.45	2.44	2.65*
- at end of recovery period	2.48	—	—	2.27	2.30	—	—	2.47
Albumin (g/l)								
- at end of treatment period	34.6	34.7	35.5	36.4*	43.0	41.4	43.4	42.1
- at end of recovery period	40.6	—	—	39.4	47.2	—	—	46.2
A1 globulins (g/l)								
- at end of treatment period	10.4	9.8	9.6	9.1*	5.9	7.5*	7.5*	7.4*
- at end of recovery period	9.8	—	—	10.4	7.4	—	—	7.9
A2 globulins (g/l)								
- at end of treatment period	2.7	2.9	2.6	2.4*	2.9	2.9	2.5*	2.7
- at end of recovery period	2.7	—	—	2.9	2.2	—	—	2.1

From Tennekes et al. (1987)
* $P \leq 0.05$ (Dunnett)

An increased urinary output and a corresponding decrease in specific gravity were observed at the high dose. These findings, which were reversible, may reflect an increased fluid intake related to treatment. A slight enlargement of the kidneys was noted macroscopically for high-dose animals of both sexes. This effect was more marked in males than in females; however, it was fully reversible in male rats but not in females. The slight increment noted for absolute adrenal weights in the high-dose males as well as the slight increment in the liver to body weight ratios noted for high-dose females were considered to be incidental findings and within the range of biological variation. No further gross pathological changes were detected, and histopathology did not demonstrate any effect.

The NOAEL was 400 ppm (equal to 25.3 and 28.9 mg/kg bw per day for males and females, respectively), and a QA statement was attached (Tennekes et al., 1987).

Dogs

In a subchronic study, bentazone (purity not specified) was administered to three male and three female Beagle dogs (10–12 months of age and weighing between 9 and 10 kg [males] and

between 8.4 and 9.4 kg [females]) per group for a period of 3 months in the diet at 0, 100, 300, 1000 or 3000 ppm (equal to 0, 4.0, 12.0, 39.6 and 113.8 mg/kg bw per day for both sexes). The animals were housed singly under controlled conditions and received a daily ration of 40 g/kg bw commercial diet (Altromin H). Water was available ad libitum.

Dietary concentrations of 100 and 300 ppm were tolerated without any symptoms. At 1000 ppm, one of the six animals displayed a slight but increasing sedation during the last 2 weeks of the study. The same dog developed an ulcer on the left hind leg. The surrounding area was affected by alopecia, and the ulcer had not healed at the end of the study. No other pathological changes were noted at this dose level. At 3000 ppm, three of the six animals died in a coma, preceded by agonal spasms in two cases. Signs of toxicity noted were sedation, attacks of superactivity, ataxia, prostration, loss of rising reflex and tremors. The sedative effect was seen first between the 2nd and 4th weeks of treatment in all high-dose dogs. It appeared 10–60 minutes after feed and compound intake. Its duration increased from about 5 hours up to 24 hours at the end of the treatment period. The three male dogs in this group vomited from time to time. In the second half of the study, all animals had increasingly severe diarrhoea, in some cases with visible blood. Anorexia was observed during the whole study period. At first, the feed consumption was only retarded, but later, the amount consumed was reduced. All animals lost weight. At 3000 ppm, all six animals had bilateral haemorrhagic conjunctivitis, mostly in a mild form. Male animals exhibited ulcerative stomatitis. One male had ulcerations surrounded by areas of alopecia on the right paw, the right ear and left of the umbilicus. The recuperative powers of the animals appeared to have been diminished, as none of these inflammatory changes healed by the end of the trial. Oedema in the thoracic region was observed in one male. Erythrocyte count, haemoglobin and haematocrit were reduced in the high-dose group. Blood sedimentation and blood coagulation were retarded in this group, and the platelet count was reduced. Furthermore, there was increased activity of several serum enzymes, such as alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase, increased urea and bilirubin contents and reduced albumin and total protein concentrations in the blood. Albuminuria and ketonuria occurred more frequently in this group.

At 3000 ppm, necropsy in all dogs revealed pale liver. Pale kidneys were noted in one dog. The males also had heavily marked lobes of the liver, and one had gastric ulcers. Swelling of the thoracic region was noted in one male and one female animal. Liver, kidneys and adrenals were distinctly enlarged. Relatively high weights were also recorded for spleen, lungs, thymus, thyroid and brain. At histopathology, there were some compound-induced changes, mainly consisting of severe congestive symptoms and necrotic congestion of the liver lobe centres. Marked fatty degeneration was observed in five dogs. These liver findings were in keeping with hypoxic damage to liver parenchyma. Furthermore, extramedullary haematopoiesis in the spleen was noted. However, the bone marrow showed a normoplastic picture. Droplets of fatty degeneration in the ventricular myocardium and albuminous swelling of the renal tubules were observed. Histological examination of the organs from the animals of the lower dose groups revealed no compound-induced pathological changes.

The highest dose of 3000 ppm was severely toxic to the dogs and was lethal in three out of six animals. The maximum tolerated dose was clearly exceeded. Anaemia and disturbed blood coagulation as well as signs of kidney and liver damage were noted, confirming the findings obtained in other species. The clinical findings, such as cachexia, conjunctivitis, stomatitis and oedema, were assessed to be secondary effects of the severe intoxication. The histopathological findings were considered to reflect chronic hypoxidosis caused by treatment.

The NOAEL was 300 ppm (equal to 12.0 mg/kg bw per day), on the basis of sedation and ulceration and alopecia in the leg of one dog at 1000 ppm (equal to 39.6 mg/kg bw per day) (Leuschner et al., 1970; Leuschner & Otto, 1972, 1973).

In a 1-year study in Beagle dogs (aged 5–7 months and weighing around 7.1–9.4 kg [males] and 7.0–9.3 kg [females]), four groups of six males and six females were given diets containing bentazone (batch N 187, purity 97.8%) at a concentration of 0, 100, 400 or 1600 ppm (equal to 0, 3.2, 13.1 and 52.3 mg/kg bw per day) for 52 weeks.

Treatment-related clinical signs were restricted to a few individual dogs of the highest dose group. One male dog of the highest dose group showed slight, but persistent, weight losses during the first half of the dosing period. Body weight during the second half of the study showed only slight fluctuations. From weeks 19 to 52, this dog appeared emaciated and dehydrated, although feed consumption throughout the treatment period remained maximal. Another male dog of the same group exhibited frequent diarrhoea. This was observed an average 1–2 times per week and persisted throughout the treatment period. The highest frequency was observed between weeks 3 and 7, when diarrhoea was recorded virtually every day. On days 27, 28, 29 and 40, the faeces appeared red, probably due to the presence of blood. A moderate decrease in activity and the pale appearance of the mucous membranes were first recorded for this dog on day 42. At this time, haematological investigations revealed a marked anaemia. Treatment was withdrawn between days 44 and 49, and the animal was offered control diet. Feed consumption remained maximal at all times. During week 8, an appreciable increase occurred in the values recorded for red blood cell parameters. Between days 43 and 53, the decrease in activity was scored as slight, whereas the mucous membranes of the mouth became increasingly pale. From day 54, however, the oral mucous membranes were scored as only slightly pale, and by day 74, colour had returned to normal. A third high-dose male dog had slight to marked hyperaemia of the skin of the ear pinnae from week 7 and of the legs and paws from week 20. Slight to marked alopecia affecting the ear pinnae, paws and head was also recorded from week 8. An improvement in the condition of the skin was evident from week 25, and complete recovery was seen by the end of week 27. In a high-dose female dog, a marked reduction in feed consumption was recorded during week 3. This was associated with the appearance of diarrhoea, with the faeces thought to contain blood. A diagnosis of gastroenteritis was made, and the animal was treated with antibiotics on days 16, 17, 19, 25, 26 and 27. A subsequent improvement in the condition of this dog was observed, and normal feed consumption was recorded from week 8. No mortality was observed throughout the study.

There were no ophthalmoscopic changes that could be related to treatment. Auditory perception was also unaffected. Occult blood was not detected in the faeces of any of the dogs of the control and high-dose groups tested during week 14 of treatment. Body weight development was not impaired at any dose, based on the mean values; however, body weight development was impaired in a few individual dogs. Overall mean body weights of the dosed animals were not statistically different from those of controls.

Examination of group mean haematological data recorded at 13, 26 and 52 weeks did not reveal any findings of toxicological significance. However, some remarkable changes were noted in individual animals. During week 7, deterioration in clinical condition was apparent for a 1600 ppm male. Off-schedule haematology revealed a marked anaemia as well as thrombocytosis, reticulocytosis, leukocytosis and changes in red blood cell morphology. A slight increase in the partial thromboplastin time was also recorded (up to 50%). Further haematological investigations during week 8 (following a 6-day period without treatment) showed an increase in the erythrocyte count, haemoglobin concentration and haematocrit values over those recorded during the previous week, although all values remained lower than those recorded pretest. A reduction in platelet, leukocyte and reticulocyte counts was also noted, and the partial thromboplastin time recorded on this occasion appeared normal. At 13 weeks, slight reductions were still apparent in the haemoglobin concentration and haematocrit value, although the platelet, erythrocyte and leukocyte counts all appeared normal. The prothrombin and partial thromboplastin times recorded for this dog, however, were both longer than those seen in other animals at 13 weeks. At 13 weeks, evidence of slight anaemia was also recorded for a female from the 1600 ppm group. This was characterized by a depression of the erythrocyte count, haemoglobin concentration and haematocrit value, with increased mean cell volume and decreased mean corpuscular haemoglobin concentration. Increased number of nucleated erythrocytes, abnormal red cell morphology (slight anisocytosis), increased thromboplastin and partial thromboplastin times, an increase in segmented neutrophils and a decrease in lymphocytes were also noted at this examination. The values for these parameters were within expected limits at 26 and 52 weeks. Increasing thromboplastin and partial thromboplastin times were recorded for another male from the 1600 ppm group as the study progressed. Slightly increased partial thromboplastin time only was recorded for two 1600 ppm females at week 13 in comparison with their pretest values. The

findings for these four high-dose dogs contributed towards the significantly higher partial thromboplastin times recorded for both males and females of the 1600 ppm group at week 13. However, values for the remaining dogs in this group were similar to or lower than those recorded pretest, and therefore this effect cannot clearly be attributed to treatment (Table 8).

Table 8. Selected haematological findings in a 1-year study in dogs

Dietary concentration (ppm)	Period of observation	PT (s)		PTT (s)		RBCs (millions/ mm^3)		Reticulocytes per 1000 RBCs		HCT (l/l)		Hb (mmol/l)	
		M	F	M	F	M	F	M	F	M	F	M	F
0 (control)	Pretreatment	6.3	6.5	10.55	10.6	6.0	6.9	0.006	0.007	0.39	0.46	8.1	9.4
	Week 13	6.5	6.5	9.9	10.2	6.2	6.9	0.001	0.004	0.43	0.48	8.6	9.6
	Week 26	6.3	6.5	9.6	10.1	6.1	6.4	0.007	0.005	0.41	0.43	8.6	9.2
	Week 52	6.7	6.8	10.3	9.8	6.5	6.3	0.004	0.003	0.42	0.43	9.1	9.2
100	Pretreatment	6.4	6.2	11.0	10.9	6.4	6.8	0.009	0.005	0.43	0.45	8.6	9.3
	Week 13	6.5	6.5	10.4	10.6	6.6	6.9	0.004	0.003	0.45	0.47	9.3	9.6
	Week 26	6.3	6.3	10.7	9.8	6.5	6.3	0.004	0.005	0.42	0.42	9.2	9.0
	Week 52	6.6	6.7	10.3	10.05	6.8	6.8	0.005	0.005	0.47	0.46	9.8	9.8
400	Pretreatment	6.5	6.05	10.5	11.1	6.3	6.7	0.007	0.004	0.42	0.44	8.5	9.3
	Week 13	6.6	6.3	10.6	10.7	6.2	6.9	0.003	0.001	0.42	0.48	8.6	9.7
	Week 26	6.4	6.3	10.3	10.7	5.9	6.5	0.007	0.003	0.41	0.46	8.5	9.4
	Week 52	6.7	6.7	9.8	10.4	6.4	6.8	0.004	0.003	0.45	0.48	9.2	10.0
1600	Pretreatment	6.25	6.4	11.2	11.3	6.3	6.9	0.011	0.005	0.44	0.45	8.6	9.5
	Week 7[a]	6.3	—	14.0	—	2.4	—	0.380	—	0.19	—	—	—
	Week 8[a]	6.4	—	11.4	—	4.0	—	0.026	—	0.31	—	—	—
	Week 13	7.45	7.1	11.7	11.8	6.25	6.8	0.002	0.001	0.42	0.45	8.4	8.8
	Week 26	6.4	6.7	10.9	10.6	6.2	6.7	0.003	0.003	0.41	0.44	8.5	9.1
	Week 52	6.9	7.1	10.9	10.6	6.5	6.6	0.003	0.003	0.45	0.44	9.4	9.3

From Allen et al. (1989)

F, female; Hb, haemoglobin; HCT, haematocrit; M, male; PT, prothrombin time; PTT, partial thromboplastin time; RBC, red blood cells

[a] Only one male animal No. 21 was subjected to test because of its illness.

Examination of the clinical biochemistry and urine analysis data recorded at 13, 26 and 52 weeks did not reveal any findings of toxicological significance. Organ weights were unaffected by treatment with bentazone. No treatment-related gross pathological findings were observed. Most macroscopic findings were unremarkable and among those normally recorded in this age and strain of dog.

As a histopathological examination of the bone (sternum) was required by the protocol but omitted from the original examination, an amendment of the pathology report (Allen, 1989), including evaluation of the bone (sternum), was made. All pathological findings recorded, including some minor testicular alterations in five dogs that received the test article, were of a spontaneous nature common to dogs of this age and strain. There was no evidence of abnormal histopathological findings resulting from treatment with bentazone technical.

The NOAEL for the study was 400 ppm (equal to 13.1 mg/kg bw per day), on the basis of clinical signs, weight loss and anaemia at the highest dietary concentration of 1600 ppm (equal to 52.3 mg/kg bw per day). The study was GLP compliant, and a QA statement was attached (Allen et al., 1989).

(b) Dermal application

Rabbit

The 21-day dermal toxicity of bentazone technical (purity not given) was tested in New Zealand White rabbits (weight between 2.3 and 3.1 kg) with dermal doses of the test substance of 0, 250, 500 or 1000 mg/kg bw applied daily for a period of 8 hours to the intact and scarified skin of six animals of each sex per dose for a period of 21 days. The control animals were treated in the same way with tylose at a dose of 2 ml/kg bw. The animals with intact skin (i.e. half of the total) were kept under observation for a further 21 days after termination of treatment. Behaviour and feed and water consumption were checked daily. Body weights were determined weekly. Clinical, clinicochemical, haematological, gross pathological and histopathological examinations as well as urine analysis were carried out. Very slight, transient erythema was detected on the intact and scarified skin, the latter being slightly more affected. However, in no case did the reactions exceed those of the control animals. More extensive skin injuries, such as oedema and necrosis, were not observed. The test and control animals showed no differences during the withdrawal period. Behaviour, condition of coat, feed and water consumption, body weight gain, haematological and biochemical tests, urine analysis, gross pathological findings and organ weights at necropsy after 3 or 6 weeks of testing were similar for treated and control animals. Histological investigations carried out after 3 weeks on the animals with scarified skin and after a further 3 weeks' observation on the animals with intact skin revealed in isolated cases negligible inflammatory infiltration on the application site and on the untreated skin. No significant differences could be observed between the treated animals and the control animals. The isolated and very slight findings in other organs can be classified as spontaneous pathology.

The NOAEL for dermal toxicity was above 1000 mg/kg bw per day for male and female animals. The study was conducted prior to implementation of GLP (Leuschner et al., 1971).

In another study, bentazone (batch N 187, purity 97.8%) was applied daily for 6 hours to the clipped intact dorsal skin of New Zealand White rabbits (mean weight 2.29 kg for males and 2.27 kg for females; five of each sex per dose) over a period of 3 weeks using a semi-occlusive dressing. The doses were 250, 500 and 1000 mg/kg bw per day. A control group (five of each sex) was treated with solvent (0.5% aqueous carboxymethylcellulose). Feed consumption was determined once a week over the course of 1 day. Body weight was determined weekly. The animals were carefully inspected twice daily (before and after exposure). Skin findings were recorded daily (about 30–60 minutes after removal of the dressing). At the end of the study, clinicochemical and haematological examinations were carried out. All animals were assessed by gross pathology. Subsequently, a histopathological examination was carried out. After a thorough assessment (clinical examination, clinical chemistry, haematology and pathology), the dermal application of bentazone did not lead to any substance-related findings at the doses tested. Some changes were attributed to an infection by coccidia.

The NOAEL was greater than 1000 mg/kg bw per day for both sexes of rabbits. The study was GLP compliant, and a QA statement was attached (Schilling & Hildebrand, 1988).

Bentazone (batch N 194, purity 97.64%) was administered dermally to SPF New Zealand White rabbits (five of each sex per dose) for 21 consecutive days at a dose of 0 (solvent control), 250, 500 or 1000 mg/kg bw per day. The test material was applied for 6 hours/day as 0.5% aqueous Tylose CB 30.000 solution (cleaned sodium carboxymethylcellulose in distilled water) under semi-occlusive dressing covering at least 10% of the body surface. The animals were housed singly under controlled conditions, and each received a daily ration of about 130 g of standardized diet. A daily ration of approximately 250 ml/animal was available as drinking-water. The test substance preparations were made up each workday immediately before application. Clinical observations were made twice daily. A check for skin findings was carried out daily about 30 minutes after removal of the dressing. Feed consumption and body weight were recorded weekly. Clinicochemical, haematological (including clotting analysis for thromboplastin time), gross pathological and histopathological examinations were carried out at the end of the study.

There were no deaths during the study period. No clinical signs of systemic toxicity were observed. No treatment-related differences in feed consumption were noted during the study. Body weight in test animals was comparable with that seen in controls. No signs of irritation on the treated skin could be observed in all animals of the test groups. The treated skin of these animals was discoloured (yellow) by the test substance. Adhesive tape caused mechanical skin lesions beside the treated area. Haemorrhagic round areas with sharp margins and crateriform retractions were observed on the clipped dorsal area of all animals used as controls (solvent control). No treatment-related effects on clinical chemistry or haematology values were apparent in males or females. No pathomorphological findings considered to be treatment related were diagnosed. No treatment-related significantly different mean absolute or relative weight parameters and no treatment-related gross lesions or microscopic findings were detected. No treatment-related skin changes were detected.

The NOAEL for dermal toxicity (local and systemic) of bentazone was greater than 1000 mg/kg bw. The study was GLP compliant, and a QA statement was attached (Kirsch & Hildebrand, 1993).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

The chronic toxicity of bentazone was studied in Swiss Webster mice. Fifty animals of each sex were fed with diet containing bentazone (batch number and purity not given) at a concentration of 0, 100, 350 or 1600 ppm (equal to 0, 15, 52 and 237 mg/kg bw per day, respectively; means for males and females) for a period of 18 months. Five mice of each sex per dose group were subjected to interim sacrifice after 12 months. The animals were housed four per cage under controlled conditions and received standardized diet and water ad libitum.

More than 50% of the animals in the test and control groups died in the course of the study. Female in the highest dose group exhibited significantly reduced feed consumption and lower body weights. Males also showed a decrease in body weight and feed consumption after 18 months, but statistical significance was not reached.

Substance-induced gross pathological changes were not noted. At terminal sacrifice, mean absolute brain and liver weights in females of the 1600 ppm group were increased. In males, the absolute spleen weight was decreased. At this top dose level, the relative organ weights of brain and liver in both sexes and kidney and heart in females were increased. In males, the relative spleen weight was decreased. No substance-induced histological changes were found. No differences in the tumour incidence were observed between treated and control animals.

As the administration of bentazone led to an impairment of body weight gain and feed consumption as well as to organ weight changes at the highest dose level of 1600 ppm (equal to 237 mg/kg bw per day), the NOAEL was 350 ppm (equal to 52 mg/kg bw per day) for both sexes. The mortality rate was above 50% in all groups, and therefore no conclusive assessment of the observed effects is possible. The study was not GLP compliant, as it was generated prior to the implementation of GLP (Welsh et al., 1974).

The carcinogenicity of bentazone was studied in mice of the CFLP strain (hysterectomy-derived strain). Four groups, each with 40 animals of each sex, were fed with a diet containing bentazone (batch no. p.195.75, purity not given) at a concentration of 0, 100, 350 or 1600 ppm (equal to 0, 8.4, 29.7 and 138.4 mg/kg bw per day for males and 0, 9.5, 34.3 and 152.9 mg/kg bw per day for females, respectively) for a period of 82–95 weeks. If a survival rate of 25% was attained in a control or treated group (i.e. a minimum of 10 males and 10 females), all groups of that sex were killed and necropsied. If the high dietary group reached the 25% survival point first, this group was killed and necropsied, the remaining groups being treated as scheduled. Therefore, all surviving male mice receiving 1600 ppm were sacrificed after 82 weeks, and all the other male groups were terminated after 88 weeks of treatment. The female groups were maintained up to 95 weeks. The animals were caged under controlled conditions and had free access to water and standardized diet. Clinical

observations were made daily. Feed consumption was recorded weekly, and body weight was determined weekly for the first 3 months and every 2 weeks thereafter. Clinicochemical and haematological examinations were not carried out. At termination, all surviving animals were sacrificed and examined gross pathologically. Gross lesions as well as liver, spleen, lymph nodes, adrenals, thyroid, ovaries and pineal body were examined histopathologically. Mortality rates were compared between the groups using stratified contingency tables. Student's *t*-test was applied to assess the significance of intergroup differences in body weight, feed intake and water intake data.

No substance-induced findings in clinical parameters were found. The number of deaths among treated mice was similar to that of the controls, with the exception of males receiving 1600 ppm, for which there was an increased incidence of mortality during weeks 79–82. At week 78 (the required duration of a carcinogenicity study in mice), survival in the males had declined to 27, 21, 30 and 23 animals in the control, 100, 350 and 1600 ppm groups, respectively. Among females, 29, 31, 26 and 18 animals were still alive in the respective groups. Thus, at 1600 ppm, mortality had reached 55% in females at this time. However, as no macroscopic or microscopic changes were noted that could be attributed to treatment in the animals dying intercurrently, the higher mortality rate was not assessed as being substance induced. Feed consumption and body weight gain of treated animals were similar to those of control animals. No substance-induced changes were found in any gross pathological or histopathological parameters.

Under the conditions of this study, no carcinogenic effect was found, and the NOAEL was 1600 ppm (equal to 138.4 mg/kg bw per day for males and 152.9 mg/kg bw per day for females), the highest dose tested. However, the scientific value of this outcome is limited because of the insufficient number of animals on study and the high overall mortality. The study is not GLP compliant, as it was generated prior to the implementation of GLP (Hunter et al., 1978).

Bentazone (batch N 169; purity 93.9%) was administered to B6C3F1 mice (33 days old, weighing around 20.6 g [males, mean] and 16.9 g [females, mean]) at a dietary concentration of 0, 100, 400 or 2000 ppm (equal to 0, 12, 47 and 242 mg/kg bw per day for males and 0, 12, 48 and 275 mg/kg bw per day for females, respectively) for 6, 12 or 24 months. Bentazone was administered to groups of 70 mice of each sex at a dietary concentration of 0, 100, 400 or 2000 ppm for about 6 or 12 months (satellite groups; 10 animals of each sex per dose) and 24 months (main groups; 50 animals of each sex per dose). The animals were examined for morbidity or mortality twice daily. If animals were in a moribund state, they were sacrificed and necropsied. Animals found dead were necropsied as soon as possible. Signs (appearance and general behaviour) were checked daily. Palpations on skin and abdominal organs were performed once per week. The body weight of the animals was determined at the start of the treatment (day 0), at weekly intervals thereafter and prior to necropsy. At the end of the administration period, the animals were sacrificed.

Feed consumption was determined for 10 mice of each sex per group of the main test group. From week 41 onward, measurements were done for 20 mice of each sex per group to avoid group sizes of less than 10 due to death of mice. The eyes of all surviving animals were examined at the end of their administration period for any changes using the naked eye, an ophthalmoscope and a funduscope. Atropine was dropped into the eye 5–10 minutes before the examination. The haematological and clinical chemistry parameters were determined for 10 animals (8 or 9 in some groups after 6 or 12 months) per test group after their respective dosing period (17 for haematology after 24 months). Urinary parameters were determined in 10 animals (8 or 9 in some groups after 6 or 12 months) of each sex per test group after 6, 12 and 24 months of administration.

All animals—if not found dead—were sacrificed, and exsanguinated animals were necropsied and assessed by gross pathology. Animals that died intercurrently were necropsied as soon as possible after death and assessed by gross pathology. The organs were sampled, weighed and examined histopathologically. Tumours in mice examined were classified according to IARC (1979).

There were no remarkable findings in the 6- and 12-month groups. In the 24-month main group, various findings common to both sexes were noted. They comprised palpable masses in the

abdomen and dyspnoea considered to be caused by tumours in the liver, lung and haematopoietic tissues. Signs common in agony, such as lack of vigor, emaciation, reduced skin temperature, pallor in the auricles and limbs, tachypnoea, systemic cyanosis and abdominal inflation, were also observed. However, those incidences did not indicate an effect of treatment.

No deaths occurred in the 6-month test, except for a male of the control group. In the 12-month group, mortality was noted in one male in each of the control and 2000 ppm groups and in one, two and one female of the 100, 400 and 2000 ppm groups, respectively. The cumulative numbers of deaths in the 24-month test were 14 (28%), 14 (28%), 15 (30%) and 20 (40%) in the control, 100, 400 and 2000 ppm groups, respectively, for males and 10 (20%), 9 (18%), 13 (26%) and 15 (30%), respectively, for females. The mortality rates of the treated groups are not considered to be different from control values.

No treatment-related ophthalmoscopic findings were noted in the animals sacrificed after 6, 12 or 24 months.

Body weight development in the 100 and 400 ppm animals was unaffected. In the 2000 ppm males, a transient minor but statistically significant suppression of body weight gain was noted up to week 23 and again at weeks 69 and 73. No effects were noted in the females of the high-dose group.

No differences relative to controls in feed consumption or feed efficiency or water intake were seen in any of the treated groups. Occasional variations in a single week were not considered to be of relevance. There were changes in the haematological parameters, namely reduced red blood cells and increased mean corpuscular volume in the treated females after 6 months and reduced white blood cells in females after 12 months. However, there was no trend that confirmed these findings on other occasions, and therefore they are not considered to be treatment related. There was a prolonged prothrombin time for the males of the 400 and 2000 ppm groups in the 24-month test, which was taken to be the result of the toxic effect of the test substance.

Some statistically significant clinical chemistry findings were observed, notably changes in the total cholesterol concentration at months 6 and 24 and in the albumin to globulin ratio in high-dose males after 24 months. However, these deviations did not show a consistent trend, and therefore they are considered to be incidental and not treatment related.

The only notable finding in urine analysis was the increase in specific gravity after 12 months in the males fed 400 and 2000 ppm in the diet. A few other spurious findings were not considered to be treatment related.

On necropsy, no obvious changes in the organ weights and ratios in the animals sacrificed after either 6 or 12 months could be noticed. However, several significant changes (increase or decrease) were noted in the organ weights of animals sacrificed after 24 months, but none of them could be attributed to an effect of the treatment.

There were only a few gross pathological findings in the animals of the 6- and 12-month sacrifices, and they were mainly limited to single incidences. Exceptions are the hair loss on truncus or head and neck and the observation of cyst in the uterus. However, there is no consistent pattern that would indicate a relationship to treatment. There were various gross pathological findings in the 24-month sacrifice group. The findings that reached any statistical significance (from either the scheduled kill subgroup or moribund/spontaneous death) are listed in Table 9. None of them indicate a relationship with treatment.

There were several histological non-neoplastic findings in the animals sacrificed after either 6 or 12 months. Most of them were of single occurrence. Of those found with higher incidences, none appeared to indicate a treatment-related effect. They were considered to be due to physiology and ageing. The pancreas islet cell hyperplasia seen in males after 12 months was also noted after 24 months, but did not develop into neoplasms. A number of non-neoplastic findings were present in the animals of the 2-year group either killed by design or at moribund/spontaneous death; however, very few reached statistical significance. Noticeable findings were the hyperplasia of Langerhans islet cells of the pancreas in the mid- and high-dose males, an effect also seen at 12 months. However, no

neoplastic lesions developed. Ioannou (1989) reported historical control data from the Nippon Institute of Biological Sciences of 22/60 (36.7%) for males and 1/60 (1.7%) for females.

Table 9. Incidence of selected gross pathological findings (with potentially relevant statistical significance) in mice administered bentazone for 24 months (moribund and scheduled sacrifices added)

	Incidence of finding							
	Males				Females			
	0 ppm	100 ppm	400 ppm	2000 ppm	0 ppm	100 ppm	400 ppm	2000 ppm
No. of animals examined	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*
Abdominal cavity								
- retention of blood	0	5*	1	7*	3	2	4	4
Liver								
- discoloured	0	1	5*	5	3	7*	8*	4
- greyish foci	2	4	9	4	0	1	1	5*
- nodule	21	25	22	25	11	7	11*	7
- mass	6	6	8	9	2	0	3	2
Spleen								
- distinct follicles	1	3	9*	1	9	10	8	8
Thymus								
- atrophy	13	16	13*	19	7	4	9	7

From Takehara (1984b, 1985)
* $P < 0.05$ (statistical significance from either scheduled kills or moribund/spontaneous deaths; Fisher's direct computation for probability)

Calcification of the testicular tunica albuginea and deferent canals was significantly increased in males of the 400 and 2000 ppm groups after 24 months (Table 10). Ioannou (1989) reported historical control data from the Nippon Institute of Biological Sciences of 5/50 (10%) and mentioned another report with higher incidences of slight severity. The lesion was not found after 6 and 12 months. Although its pathological development is unknown, it is considered to be treatment related in view of the dose–effect relationship. Spermatogenesis of these calcified testes was normal. Besides these lesions, there were lesions that increased or decreased significantly in each treated group compared with the controls, but all of them were either secondary lesions caused by tumours or ageing lesions unrelated to the test substance.

Additional histopathological investigation into the salivary gland and mammary gland was taken from the long-term feeding study by the Nippon Institute of Biological Sciences. All of the tissues from the main study were available for evaluation. These findings were reported in detail in Yamate (1988).

Bentazone was not carcinogenic, nor did it produce any other adverse effects on the mammary gland or salivary gland. One adenocarcinoma of the mammary gland was found in a female receiving 400 ppm, which was killed at the end of 24 months. Leukaemic cell infiltration was found sporadically in salivary glands and mammary glands; there was a non-significant increase in dosed females, which was considered to be spontaneous or incidental. Lymphocytic aggregation (perivascular) in the salivary glands was frequent; lymphocytes appeared normal. For male mice sacrificed moribund or found dead, the incidence of lymphocytic aggregation (perivascular) in the salivary glands in the 2000 ppm group (17/21) was significantly ($P < 0.05$) greater than in comparable

controls (6/14), but for mice sacrificed at termination, there was no increase. This finding was not considered to be of biological importance.

Table 10. Incidence of selected non-neoplastic histopathological findings (with potentially relevant statistical significance) in mice administered bentazone for 24 months

	Incidence of finding							
	Killed by design				Terminated or found dead			
	0 ppm	100 ppm	400 ppm	2000 ppm	0 ppm	100 ppm	400 ppm	2000 ppm
24-month sacrifice males								
No. of animals examined	*36*	*36*	*35*	*29*	*14*	*14*	*15*	*21*
Liver								
- haemorrhage	0	1	0	2	1	3	2	10*
Spleen								
- extramedullary haematopoiesis, slight	1	0	0	1	0	5*	1	2
Heart								
- haemorrhage	0	0	0	0	0	0	0	6*
Pancreas								
- hyperplasia of islet cells	7	8	15*	12*	2	3	4	10*
Brain								
- vacuolization in white matter	1	32***	2	2	0	1	0	0
Testis								
- calcification	2	5	12**	24***	0	1	0	11***
24-month sacrifice females								
No. of animals examined	*40*	*41*	*37*	*35*	*10*	*9*	*13*	*15*
Lung								
- haemorrhage	0	1	1	1	0	0	5*	1
Lymph nodes, mesenteric								
- red blood cell infiltration in sinus	0	0	5*	0	0	0	1	0

From Takehara (1984b, 1985)
* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$ (Fisher's direct computation for probability)

The tumour incidence in most organs was low and/or did not reach statistical significance or did not suggest a treatment-related effect due to a lack of a dose–effect relationship. The notable exception is the liver. In the original assessment, the incidence of liver tumours (neoplastic nodules and hepatocellular carcinoma) in each group was 52–70% for male mice and 10–26% for female mice. The corresponding historical control incidence is quoted as approximately 80% for males and 13% for females (Ward et al., 1978), and ranges from the literature are given as 7–55% for males and 0–21% for females (Tarone, Chu & Ward, 1981). Further, it is noted that the sums of neoplastic nodules and hepatocellular carcinomas for each group were not statistically significantly different from the controls. Further, in the 6- and 12-month tests, swelling of hepatocytes, degenerative focus or hepatocellular tumours were not observed in the higher dose groups. The conclusion is that the hepatic tumours observed are not due to the treatment with bentazone. In Ioannou (1989), slightly different control data are quoted, yet the conclusion that bentazone is not carcinogenic in the livers of

B6C3F1 mice is supported. It is also mentioned that these control data are higher than data published by Haseman, Huff & Boorman (1984) based on 2300 mice.

The hepatic tumours were reviewed after completion of the report and reassessed according to Vesselinovitch, Mihailovich & Rao (1978). Hyperplastic and adenomatous nodules were classified as non-carcinomatous nodules, and trabecular nodules were classified as carcinomas. There were no significant differences in the incidences of non-carcinomatous nodules and carcinomas between the controls and any of the treated groups, except non-carcinomatous nodules in the female mice receiving 2000 ppm. Thus, the conclusion regarding the absence of an oncogenic effect in the liver was confirmed.

Other tumour lesions found in various other organs (e.g. lung and haematopoietic system) did not show a pattern suggestive of a treatment-related effect, and the incidences were not significantly different from controls. The total number of tumours, number of tumour-bearing mice and number of tumours per mouse in the treated groups were all similar to those of the controls and without any significant difference. Thus, no oncogenic potential was found in this study.

Bentazone administered to mice at dietary concentrations of 0, 100, 400 and 2000 ppm for 2 years resulted in a transient impairment of body weight development in males at 2000 ppm. Bentazone caused a prolongation of the prothrombin time in males at 400 and 2000 ppm, which is in agreement with similar findings in the short-term studies of toxicity in mice and rats (see above). The gross pathological examination showed various lesions in the liver, spleen and thymus in animals of all the treatment groups and the control group. These lesions occurred in some cases significantly more frequently only in the two intermediate dose groups and showed no dose–response relationship. They were assessed as being age induced and not related to the test substance.

Testis histology revealed an increased incidence of calcification of the testicular tunica albuginea and deferent canals in the 400 and 2000 ppm males. In view of historical control data, this change is a very common lesion in aged mice, including the strain used in this study. There was, in addition, no evidence of any other adverse effect on testes or on male reproductive performance obtained in any study on bentazone.

There were no neoplastic changes indicative of an effect of bentazone; however, bentazone may have slightly increased proliferative lesions in the liver of female mice. Thus, the compound did not show an oncogenic potential.

The NOAEL was 100 ppm (equal to 12 mg/kg bw per day in both sexes), based on prolongation of prothrombin time and increased incidence of calcification of the testicular tunica albuginea and deferent canals in males at 400 ppm (equal to 47 mg/kg bw per day). The study was conducted according to the principles of GLP, and a QA statement was attached (Takehara, 1984b, 1985; Carlton et al., 1987; Millar, 1987; Butler, 1988; Yamate, 1988; Ioannou, 1989).

Rats

In a chronic toxicity study, bentazone (batch and purity not given) was administered to Sprague-Dawley rats (50 of each sex per group) via a diet containing 0, 100, 350 or 1600 ppm (equal to 0, 5, 17 and 76 mg/kg bw per day, respectively; means for males and females) for 2 years. The animals were housed singly under controlled conditions and received standardized diet and water ad libitum. Clinical observations were made daily. Feed consumption was recorded weekly for the first 2 months and then every 2 weeks. Body weight was determined weekly for the first 2 months and monthly thereafter. Ophthalmoscopy was carried out prior to the beginning of the study and after 6, 9, 12, 18 and 24 months. Haematological, clinical chemistry and urine analysis parameters were determined in 5 animals of each sex per dose group after 3, 6, 9 and 12 months and in 10 animals of each sex per dose group after 18 and 24 months. After 12 months, five animals of each sex per dose group were sacrificed and necropsied. All the sacrificed animals were subjected to a gross pathological examination. All sacrificed animals of the control group and of the highest dose group and 10 animals of each sex for each of the other dose groups were examined histopathologically. However, blood coagulation parameters were not investigated. For statistical calculations, various

methods were applied, including Bartlett's test for homogeneity of variances, analysis of variance, Duncan's multiple range test and Wilcoxon or Mann-Whitney rank sum test.

Mortality was not affected by the substance administration. Statistical evaluation of body weight revealed a significant decrease at 1600 ppm in both sexes due to a diminished body weight gain in the 2nd year of the study. Similarly, mean feed consumption was reduced at the top dose level in the 2nd year. No treatment-related findings in haematology, clinical chemistry or ophthalmoscopy were noted. At 1600 ppm, the organ weights of kidney, liver and spleen in both sexes and brain and heart in females were increased. The organ to body weight ratios of kidney, liver and spleen were increased in both sexes. Females exhibited an increased brain and heart to body weight ratio. No statistical significance was shown in any of the other three dose groups. Tumours that occurred were examined histologically and did not reveal any signs of malignancy. Statistical analysis of tumour incidence did not reveal any significance among the groups tested. No substance-induced histopathological changes were noted.

In view of the above observations, the NOAEL for chronic toxicity was 350 ppm (equal to 17 mg/kg bw per day for both sexes), based on decreased feed consumption and body weight gain and increased organ weights at the highest dose level. The study is not GLP compliant, as it was generated prior to the implementation of GLP (Cannon et al., 1974).

Bentazone (batch N 169; purity 93.9%; free acid) was administered to groups of 70 Fischer F344 Du/Crj (SPF) rats (34–35 days old, weighing between 77 and 108 g [males] and 70 and 89 g [females]) of each sex at a dietary concentration of 200, 800 or 4000 ppm for about 6 or 12 months (satellite groups; 10 animals of each sex per dose) and 24 months (main groups; 50 animals of each sex per dose). At the end of the respective administration period, the animals were sacrificed.

The mean compound intakes corresponded well with the nominal dose levels and were calculated for the periods of 1–26, 1–52 and 1–104 weeks (Table 11). Both sexes ingested more of the compound when they were younger, and the intakes decreased with age.

Table 11. Mean compound intake over the duration of the 2-year study in rats

Sex	Period (weeks)	Compound intake (mg/kg bw per day)		
		200 ppm	800 ppm	4000 ppm
Males	1–26	12	47	233
	1–52	9	39	197
	1–104	9	35	180
Females	1–26	14	55	274
	1–52	12	48	249
	1–104	11	45	244

From Takehara (1984a)

The animals were examined for their general state (appearance and behaviour), morbidity or mortality twice daily on working days and once daily on weekends and public holidays. If animals were in a moribund state, they were sacrificed and necropsied. Animals found dead were necropsied as soon as possible. Palpations on skin and abdominal organs were performed once per week. The body weight of the animals was determined at the start of the treatment (day 0), at weekly intervals thereafter and prior to necropsy. Feed consumption was determined on 10 rats of both sexes in each group of the main test group. From week 28 onward, measurements were done on 20 rats of each sex per group to avoid group sizes of less than 10 due to death of rats. Water intake was determined for 10 rats of both sexes in each group of the main test group. From week 28 onward, measurements were done on 20 rats of each sex per group to avoid group sizes of less than 10 due to death of rats.

The eyes of all surviving animals were examined at the end of their administration period for any changes using the naked eye, an ophthalmoscope and a funduscope. Atropine was dropped into the eye 5–10 minutes before the examination. Blood samples were withdrawn under light anaesthesia from the descending aorta by laparatomy. The haematological and clinical chemistry parameters were determined for 10 animals (9 at 800 ppm for females after 12 months) per test group after their respective dosing period (17 for haematology after 24 months). The urine analyses were conducted for 10 animals of each sex per group after 6, 12 and 24 months of administration. All animals—if not found dead—were sacrificed by alcohol, chloroform and ether vapour anaesthesia and exsanguination. The exsanguinated animals were necropsied and assessed by gross pathology. Animals that died intercurrently were necropsied as soon as possible after death and assessed by gross pathology. The organs were sampled, weighed and examined histopathologically.

No specific condition caused by the administration of bentazone was noted in any of the groups in either sex over the 6-, 12- or 24-month period. In both treated and control groups, almost all rats survived until month 12. Mortality increased from week 81 onwards, but none of the groups showed any early deaths or high rate of death. In the 24-month group, mortalities for the 200, 800 and 4000 ppm groups ranged from 18% to 44% for males and from 30% to 46% for females, respectively. The mortalities for treated males and females were not significantly different from those of the respective controls.

No treatment-related ophthalmoscopic findings were noted in the animals sacrificed after 6 or 12 months. In the 24-month animals, cataracts were observed in males receiving 4000 ppm, but also in control males. The incidences were low in the 200 and 800 ppm groups. Retinal changes and cataracts are frequent, age-induced manifestations in the strain of rats used, with a widely variable incidence. All cataracts except one were unilateral, and all except one appeared after 24 months. Two points argue against a cataractogenic substance effect. In the case of a substance-related cataractogenic effect, bilateral cataracts would be expected. Furthermore, cataractogenic substances have been shown to predominantly affect younger animals. This is attributed, in part, to differences in the penetrability of drugs from the bloodstream through the blood–aqueous barrier into the eye, as well as to innate differences in the susceptibility of the lens itself to a cataractogenic effect of a drug. Accordingly, it seems unlikely that the unilateral cataract noted in males receiving 4000 ppm was caused by the test substance. This position was verified after a re-examination of the above-mentioned clinical and pathological findings and reinforced in the supplemental report (Takehara, 1986, based on a review performed by Butler, 1985), which states that "the age-related susceptibility of the lens to the test substance could not be demonstrated". Furthermore, significant differences in the incidence of retinal degeneration and atrophy between treatment and control animals were not detected when these findings were regarded as one type of lesion. The toxicological significance of the degenerative changes in the optic nerve remains equivocal, in particular, as a detailed examination revealing atrophy was confined to high-dose males. Furthermore, it should be taken into account that such eye findings were not noted in any other long-term or subchronic study with bentazone.

Body weight gain in the 200 ppm animals was unaffected. In the 800 ppm animals, a transient suppression of body weight gain was noted in the period between weeks 19 and 36 in males and in the separate weeks 60 and 65 in females.

The weekly body weight gains were frequently suppressed from weeks 5–6 onward in both sexes treated with 4000 ppm.

The overall body weight gain after week 104 was reduced only in high-dose males and females by about 4.7% and 7.7%, respectively. After week 12, body weight gain was suppressed in the high-dose males by about 4% and in females by about 2.6%.

No differences in feed consumption relative to controls were seen in the animals in the 200 ppm group. In the 800 and 4000 ppm groups, feed consumption tended to decrease in males and was approximately the same as in controls in females. There was no significant difference in the total average feed efficiencies for males and females between every treated group and the control group.

The water consumption of the 200 ppm males was comparable to that of controls over the whole test period. In the 800 ppm males, water consumption was increased occasionally between weeks 29 and 77. In the females of this dose group, water consumption was increased from week 29 until the end of the study. In the 4000 ppm group, water intake was increased from week 6 onwards in males and from week 17 onwards in females. Over the complete period, the mean weekly water intake in these high-dose animals was increased by about 40%.

There were a number of variations in the red and white blood cell parameters in either direction (Table 12). These changes remained within the normal range and were not considered to be treatment related. Blood platelet counts were reduced in both sexes of the 4000 ppm group as well as in males in the 800 ppm group at 6 months. The reduction in platelet counts in the low-dose group after 12 months in females was not considered to be treatment related due to the lack of a dose–response relationship.

Prothrombin time and activated partial thromboplastin time were prolonged in males receiving 4000 ppm at months 6 and 12. Activated partial thromboplastin time was prolonged in males receiving 800 ppm at month 12 and in males receiving 4000 ppm at month 24 (Table 12). Females showed a prolongation of activated partial thromboplastin time in the high-dose group after 12 months. The changes in prothrombin time in females after 24 months at the middle dose and in activated partial thromboplastin time in females in the 800 ppm group after 12 and 24 months were discussed in the report by Takehara (1984a) as not being substance related. This conclusion was further supported by Ioannou (1989), in which a statistical re-evaluation revealed no significance. Therefore, prolongations in prothrombin time and/or activated partial thromboplastin time in combination with reduced platelet counts within 6 months and prothrombin time and/or activated partial thromboplastin time within a 1-year interval suggest the presence of haemorrhagic diathesis and are considered to be the result of test substance administration. This is further supported by the observations in one dead male (#232) in the 4000 ppm group showing haemorrhagic lesions in the intraperitoneal adipose tissue and pia mater of the rhinencephalon. No female died in the first 6 months, but in the second 6-month period up to 12 months, one female in the 800 ppm group (#756) died with haemorrhage in the thoracic cavity.

A number of statistically significant clinical chemistry findings were observed; however, many of them were considered as either not relevant/adverse or not treatment related. For example, the decrease in lactate dehydrogenase level in females was considered to fall within the physiological range of values and was not considered to be a morbid change.

At month 6, blood urea nitrogen concentration was increased in males in all treated groups and in the high-dose females. In the 4000 ppm group males, the albumin to globulin ratio was increased, total cholesterol concentration was decreased and glucose concentration and aspartate aminotransferase activity tended to decrease, whereas in 4000 ppm females, alanine aminotransferase activity was decreased. At month 12, in the 4000 ppm group, glucose concentration, aspartate aminotransferase activity and sodium ion concentration were decreased in males, whereas the albumin to globulin ratio and creatinine and blood urea nitrogen concentrations were increased in females. The blood urea nitrogen concentration was also increased in females receiving 800 ppm. At month 24, glucose and total cholesterol concentrations were decreased in males and females receiving 4000 ppm.

At 6 and 12 months, the urine volume was increased in both sexes within the 4000 ppm group, whereas the specific gravity decreased in parallel. In both sexes in the 800 ppm group, a volume increase coupled with a decrease in specific gravity was limited to the 6-month analysis. After 24 months, the specific gravity was decreased in both sexes in the 4000 ppm group and in females in the 800 ppm group. None of the corresponding urine volumes were increased; on the contrary, the high-dose females showed 47% reduced urine volume. A urine volume increase was apparent only in 800 ppm males, but without changes in specific gravity. The colour of the urine ranged from yellow in the controls to light yellow in the dosed groups. In the report, it was suggested that the increase in urine volume coupled with the decrease in specific gravity in the 4000 and 800 ppm groups was related to an increase in water intake. However, the decrease in urinary specific gravity in the absence

of an increase in urinary volume in females in the 200 ppm group at month 6 and in males in the 200 and 800 ppm groups at month 12 was not considered to be related to the treatment.

Table 12. Selected haematological findings (group means) in rats administered bentazone for 6, 12 or 24 months

	Month	Males				Females			
		0 ppm	200 ppm	800 ppm	4000 ppm	0 ppm	200 ppm	800 ppm	4000 ppm
RBC (10^4/mm^3)	6	845	800	808	823	842	815	821	813
	12	968	934	926	968	903	918	925	896
	24	761	619	865	857	841	859	904	834
Hb (g/dl)	6	15.0	15.2	15.4**	15.5**	14.8	15.2**	15.2*	14.9
	12	15.5	15.2	15.3	16.0*	15.2	15.3	15.5	15.6***
	24	13.5	11.3	13.6	14.5	14.4	13.7	14.3	14.4
HCT (%)	6	47.2	47.3	47.7	47.8	45.7	46.4	46.3	45.0
	12	47.9	47.4	47.5	49.0***	46.9	46.5	47.5	47.3
	24	43.3	37.5	45.0	46.8	45.1	44.6	46.4	46.3
MCV (µm^3)	6	56.1	59.3	59.1*	58.1	54.4	56.9*	56.6	55.5
	12	49.5	50.8	51.5	50.7	52.1	50.7	51.5	52.9
	24	60.1	62.0	52.2	55.0	54.3	53.9	51.7	55.7
MCH (µg)	6	17.8	19.0*	19.1*	18.8*	17.6	18.7**	18.6*	18.3
	12	16.0	16.4	16.6	16.5	16.9	16.7	16.8	17.5
	24	18.3	18.4	15.6*	17.1	17.3	16.5	15.9	17.4
MCHC (%)	6	31.9	32.1	32.3**	32.4**	32.3	32.8*	32.9***	33.0***
	12	32.3	32.2	32.2	32.6	32.4	33.0*	32.6	33.1**
	24	31.6	29.8	29.9	31.1	31.8	30.7***	30.8***	31.2*
WBC (10^2/mm^3)	6.0	47.0	56**	56**	50.0	34.0	37,0	35.0	34,0
	12.0	57.0	57.0	61.0	57.0	30.0	28,0	33.0	32,0
	24.0	121.0	121.0	98.0	48.0	56.0	69,0	37.0	31,0
Platelets (10^4/mm^3)	6	69	66	64**	61***	67	70	64	59***
	12	63	65	61	61	57	51***	58	58
	24	82	63	89	100	64	55	66	69
PT (s)	6	19.0	18.5	19.8	22.4***	15.8	15.6	15.8	15.3
	12	19.0	18.4	18.8	23.4***	16.0	16.6	16.0	16.5
	24	16.1	16.5	16.4	15.9	15.7	16.0	16.3[a]	15.2
APTT (s)	6	19.8	20.2	19.7	24.0**	16.7	15.9	16.3	17.4
	12	17.8	19.1	20.2**	22.5***	17.3	17.4	18.0[a]	18.6*
	24	15.9	16.7	17.3	18.7*	16.7	16.9	15.4[a]	17.3

From Takehara (1984a)
APTT, activated partial thromboplastin time; Hb, haemoglobin; HCT, haematocrit; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; PT, prothrombin time; RBC, red blood cells; WBC, white blood cells
* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$ (Student's *t*-test)
[a] Including corrections of Ioannou (1989).

At 6, 12 and 24 months, the pH and protein level in the urine tended to decrease in the treated rats of both sexes dose dependently. This might reflect a modification of the excretion pattern in a

dose-dependent matter, which is not solely associated with higher urinary output. A few other spurious findings were considered to be not treatment related or not adverse. These were an increase in bilirubin level in the low-dose animals of both sexes at 6 months and the tendency for a decrease in urobilinogen and ketone bodies in male and female rats at 24 months.

Organ weights, satellite groups (6-month sacrifice): A decrease in terminal body weights was observed in the high-dose animals. This decrease was statistically not significant in the males (approximately 4.7%) but significant (approximately 6.4%) in the females.

The kidney weight and the kidney to body weight ratio were increased in males and females receiving 4000 ppm, but without histopathological correlate. This effect is probably related to the higher urinary output and the reduction of body weight in the top-dose animals. The weight of the thyroid and the thyroid to body weight ratio were decreased in 800 and 4000 ppm males, and the thymus weight and thymus to body weight ratio were significantly decreased in 4000 ppm females, both organs without histopathological correlates.

Both the absolute and relative weights of the pituitary were significantly decreased in males of all treated groups at month 6, but these decreases were considered to be incidental rather than treatment related. The changes in absolute or relative weights of liver, spleen, heart, lung, testes and brain in the high-dose females or males were not accompanied by corroborative histomorphological changes, were not found in both sexes and were not found to be dose dependent. Thus, these changes were not considered to be treatment related but rather were attributed to body weight changes or biological variation. The adrenals were not affected in the interim kill groups but are listed here for better comparability with the main group animals and to put the data of the 90-day study (see above), which showed a significant increase in absolute adrenal weight in the high-dose males without histopathological correlate, into perspective. In respect of this study, these effects on adrenal weight are considered to be not substance related.

Organ weights, satellite groups (12-month sacrifice): A decrease in terminal body weights by about 7.3% was observed in the high-dose males. The kidney weight and kidney to body weight ratio were increased in males and females receiving 4000 ppm, again without histopathological correlate. It is assumed that this result is probably related to the higher urinary output and the decreased body weight. The weight of the thyroid and thyroid to body weight ratio were decreased in males receiving 800 and 4000 ppm, and the absolute thyroid weight was decreased in females receiving 4000 ppm. Histopathological examinations revealed a non-significant increase in hyperplasia of C-cells. The decrease in pituitary weight seen in males at month 6 was no longer present at 12 months. Decreases in relative and/or absolute weights of liver, spleen, heart, lungs and brain in high-dose males were considered to be secondary to the significantly lower terminal body weights. The decrease in absolute brain weight in females in all dose groups was not dose dependent or correlated with morphological changes. The weight of thymus and the thymus to body weight ratio were reduced, predominantly in males and marginally in females, each without statistical significance or dose dependency. Additionally, no histopathological change was seen in the thymus, so the significance of the weight decrease in females after 6 months is questionable. Adrenals and testes/ovaries were not affected after 12 months.

Organ weights, main group (2-year sacrifice): A decrease in terminal body weight was observed in the high-dose animals, significantly for females.

Absolute kidney weight was reduced in males at 800 ppm without a dose–response relationship. The relative kidney weight was increased in males and females receiving 4000 ppm and decreased in males receiving 800 ppm (according to the statistical re-evaluation performed on request of the United States Environmental Protection Agency [USEPA]; see Ioannou, 1989). Histopathological evaluation revealed a reduction in the severity of chronic nephropathy at higher dose levels in females, so the relevance of this effect is questionable. The absolute and relative weights of the liver were decreased in males receiving 800 and 4000 ppm, and the weight of the spleen was also decreased in males receiving 4000 ppm. These changes were discussed in the report (Takehara, 1984a) as reflecting the reduction in body weight. This is also valid for the changes in heart and brain weights in males. Weight decreases in left-side adrenals in all treated females were not

reflected in the sum of the means, were not dose related and were completely opposite to the weight increase in the low-dose males. The increases in absolute and relative testes weights in the mid-dose males as well as the absolute weight increase in the ovaries were not dose related. No significant weight effects were seen in the pituitary, thyroid or lungs. The thymus was not weighed in the 24-month group because of general age-related atrophy.

Gross pathology: There were only a few gross pathological findings in the animals of the 6-month sacrifice, and they were mainly limited to single incidences without statistical significance. Two males died prematurely; one from the 200 ppm group was killed in a moribund state, and the other from the 4000 ppm group died (#323) showing signs of bleeding, with haemorrhagic lesions in the intraperitoneal adipose tissue and pia mater of the rhinencephalon as well as anaemic appearance of major organs. In the following 6 months, spontaneous mortality occurred in a female of the control group, a male of the 200 ppm group and a female of the 800 ppm group. No findings that could be related to treatment were made. There were various gross pathological findings in the 24-month sacrifice group. Although certain findings reached statistical significance (from either the scheduled kill subgroup or moribund/spontaneous deaths), none of them indicated a relationship to treatment except for the atrophy of the optic nerve in the 4000 ppm males.

Histopathology, non-neoplastic lesions, 6- and 12-month groups: There were several histological non-neoplastic findings in the animals sacrificed after either 6 or 12 months. Most of them were of single occurrence, showed no dose–response relationship and were equally distributed between control and treated groups. Of those with higher incidences, none appeared to indicate a treatment-related effect.

Histopathology, non-neoplastic lesions, 2-year group: A number of non-neoplastic findings were present in the animals either killed by design or at moribund/spontaneous death. Histological examination (and necropsy) performed at month 24 disclosed atrophy of the optic nerves and retinal degeneration in males in the 4000 ppm group, and these lesions are deemed to be associated with cataract and the tendency of Fischer rats to develop retinal degeneration as an age-related change.

In the supplemental report (Takehara, 1986, based on a review performed by Butler, 1985), cataracts, atrophy of the optic nerve and retinal degeneration and atrophy were readdressed based on the re-evaluation of 19 control and 21 high-dose animals. The differences in numbers found in histological examination, gross postmortem examination and ophthalmoscopic examination were acknowledged, and the ophthalmoscopic examination was considered as most relevant. It was concluded that the observations in this study do not suggest a compound-related effect, although more cataracts are observed in the top-dose group (7 in control versus 18 in top-dose males).

The facts that the cataracts were all, except one, unilateral and that all, except one, appeared above 1 year of age add weight to the opinion that the effect is not substance induced. In the case of substance-induced cataracts, they would be expected to be bilateral and to occur in younger animals, in which the lens is due to a greater penetrability of drugs from the bloodstream through the blood–aqueous barrier into the eye and to an innate higher susceptibility of the lens itself to a cataractogenic effect of a drug.

By examination of rats having cataracts with or without apparent degeneration of the optic nerve, it was found that "the optic nerve atrophy coexisted with other abnormalities in the same eyeball, suggesting that there might be a relationship between optic nerve atrophy and the other lesion in the eyeball". With regard to retinal atrophy and retinal degeneration, it is argued that these should be grouped together as a single entity of retinal disorders. No statistically significant differences between incidences are then evident. The conclusion of the amendment (Butler, 1985) is that cataracts, atrophy of the optic nerve and retinal degeneration and atrophy are not related to the administration of bentazone.

Lesions observed in the liver, spleen, kidneys, lungs, adrenals and other organs could be attributed to ageing. The occurrence of these changes was more frequent in rats that died than in rats killed by design. An influence of test substance administration was not apparent in any of the organs.

The incidence of pulmonary lesions was not related to dose, and some pulmonary lesions were interpreted to have been caused by inhalation of powdered diet, because there were abundant vegetable fibres in the foci.

Histopathology, neoplastic lesions: In the initial 12 months of the study, one mesenchymal tumour was noted in one female in the 800 ppm group, an interstitial cell tumour of the testicle in two males in the 4000 ppm group and hyperplasia of chromophobic cells of the pituitary in one female in the 4000 ppm group. In the 24-month group, there were higher incidences of tumours; however, none could be associated with the treatment with bentazone. Most of the tumours occurred in the testicles, liver, adrenals, thyroids, skin, mammary glands, pituitary and uterus. In addition to tumours in these organs, atypical monocytic leukaemia showing generalized infiltration of tumour cells into various organs was noted. These manifestations could also not be related to the treatment. In the kidneys, as the primary target organ, with an organ weight increase at the top dose, no increased tumour formation was found in males or females.

Ioannou (1989) requested, in addition to the referenced historical control data in the literature, laboratory historical control data for phaeochromocytoma and uterine endometrial polyps. For phaeochromocytoma, the laboratory historical control values are stated as being 37/257 (14%) in males and 26/203 (13%) in females, which are higher than the values from two other references mentioned in the Ioannou (1989) study report. In the study, the combined incidence of phaeochromocytoma was 6, 9, 11 and 9 in males at 0, 200, 400 and 8000 ppm, which is equal to 12%, 18%, 22% and 18%, respectively. In females, the combined incidence was 0, 2, 3 and 4, which is equal to 0%, 4%, 6% and 8%, respectively. The incidence in male animals was higher than the historical control incidence, but showed no clear dose dependency, whereas the incidence in females showed dose dependency, but stayed below the historical control values. Ioannou (1989) confirmed that the observed increased incidence of phaeochromocytoma in high-dose females was not of biological importance.

For uterine polyps, the laboratory historical control incidence is 45/203 (22%), which is slightly higher than the values given in two other references. In the study, the combined incidence of uterine polyps in females was 10, 9, 18 and 12 at 0, 200, 400 and 8000 ppm, which is equal to 20%, 18%, 36% and 24%, respectively. Nevertheless, according to Iannou (1989), the data suggest that the increased incidence of uterine polyps in mid-dose females is not of biological importance.

All other neoplastic findings either were observed in single or low incidences without a relationship to treatment or were evenly distributed among all groups, including controls. They were therefore considered to be of spontaneous origin and not related to treatment. In male and female rats, the total number of tumours, number of tumour-bearing animals and number of tumours per rat were approximately the same in all groups, including controls. Thus, no oncogenic potential was found in this study.

Histopathology of decedents: Both males and females tended to die increasingly from month 13 onward, and the incidence of death for both males and females was elevated from month 19 onward. Major lesions noted in dead males include pituitary tumours, enlargement of the spleen and liver, generalized yellowish pigmentation, subcutaneous tumours in the thoracic and abdominal regions, emaciation and purulent pneumonia. In females, emaciation and purulent pneumonia were predominant causes of death, followed by pituitary tumours, enlargement of the spleen and liver, and atypical monocytic leukaemia.

Conclusions: The administration of bentazone led to a reduction in body weight gain at 4000 ppm. Although histopathological examination revealed no substance-induced changes, indications for an impairment of liver and kidney function were noted at 800 and 4000 ppm by changes in clinical chemistry and urine analysis parameters and by increased organ weights, as well as by increased water consumption at the top dose level. Blood coagulation was affected at 800 and 4000 ppm. This finding is in agreement with the results of the shorter-term studies with bentazone (see above). Decreased organ weights were assessed as being related to the decreased body weights.

Under the conditions of this study, the NOAEL was 200 ppm (equal to 9 mg/kg bw per day in males and 11 mg/kg bw per day in females), on the basis of prolonged blood coagulation and impairment of liver and kidney function at 800 ppm (equal to 35 mg/kg bw per day in males and 45 mg/kg bw per day in females). No carcinogenic effect was observed in this study.

Although the study was performed when GLP was not compulsory, it is stated in the report (Takehara, 1984a) that the study was conducted according to the principles of GLP (Takehara, 1984a; Butler, 1985, 1986, 1988; Ioannou, 1989).

2.4 Genotoxicity

Bentazone was tested for genotoxicity in 16 studies, including 10 in vitro studies and 6 in vivo studies (Table 13). There are also six genotoxicity studies in the published literature on bentazone. Bentazone gave negative results in all the studies. In one in vitro forward mutation assay in mammalian cells (HRPT test), bentazone gave a weakly positive result with mice using the S9 mix. Only two studies complied with GLP, as most of the studies were generated before implementation of GLP. In three studies, QA statements are attached. On the basis of these studies, it is concluded that bentazone is unlikely to be genotoxic. A summary of the studies described is given in Table 13.

2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

In a multigeneration study, bentazone (batch number and purity not given) was administered via the diet at a concentration of 0, 20, 60 or 180 ppm to three generations of Sprague-Dawley rats (F_0, F_1 and F_2, each with two litters). Twenty rats of each sex were used in each case. The control group received untreated diet only. Initially, all rats were housed three per cage under controlled conditions. For mating, one male and one female were placed together during the 12-hour dark period for 7 days. Once copulation had occurred, the females were separated and kept alone. The animals had free access to standardized diet and water throughout the study. The substance intake at the low dose level of 20 ppm ranged between 1.6 and 2.2 mg/kg bw per day for males and between 2.0 and 2.5 mg/kg bw per day for females. At the middle dose level (60 ppm), males received 4.5–6.4 mg/kg bw per day and females 6.4–7.3 mg/kg bw per day. High-dose (180 ppm) males received 14.1–19.4 mg/kg bw per day, and females, 19.8–21.9 mg/kg bw per day.

After 8–18 weeks of pretreatment, the F_0 animals were mated. The pups of the first litter (F_{1a}) were reared until they were 4 weeks old and then sacrificed and necropsied. The parental animals were mated again, and, at an age of 4 weeks, 20 animals of each sex per dose group were selected from the pups of the second litter and reared while being given further treatment. The remaining pups were sacrificed and necropsied. At an age of 18–29 weeks, these F_{1b} animals were mated twice, and the same procedure was carried out with the F_2 pups as with the F_1 pups.

No clinical signs of toxicity were noted. Body weight remained unaffected. Fertility and rearing behaviour of the animals were not affected. The development of the pups was comparable in all the groups. No substance-induced gross pathological or histopathological changes occurred.

Under the conditions of this study, the NOAEL for parental toxicity as well as reproductive and developmental toxicity was 180 ppm (corresponding to about 21.9 mg/kg bw per day), the highest dose tested. The study was not GLP compliant (Leuschner et al., 1973).

In a two-generation reproductive toxicity study, bentazone technical (batch no. N 187; purity 97.8%) was administered to groups of Wistar/HAN (Kfm: WIST, outbred, SPF Quality) rats (25 of each sex per group; 8 weeks old and weighing 176–224 g [males] and 138–178 g [females]) at a dietary concentration of 0, 200, 800 or 3200 ppm, corresponding to a lowest average intake of around 0, 14, 59 and 238 mg/kg bw per day, respectively, during the premating and gestation periods.

Table 13. Summary of genotoxicity studies on bentazone

Study	Strain/species	Substance; concentration/dose	Purity (%)	Result	Reference
In vitro					
Bacterial reverse mutation assay (Ames test)	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, TA1538; *Escherichia coli* WP2 hcr	Bentazone; 0, 10, 50, 100, 500, 1000 μg/plate	94	Negative (±S9)	Shirasu et al. (1976)
Bacterial reverse mutation assay (Ames test)	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538; *E. coli* WP2 hcr	Bentazone; 0, 1000, 2500, 5000, 10 000 μg/plate	94	Negative (±S9)	Moriya (1984)
Bacterial reverse mutation assay (Ames test)	*S. typhimurium* TA98, TA100, TA1537	Bentazone (77/357); 0, 3.1, 10, 31, 100, 310, 1000, 2000 μg/plate	92.5	Negative (±S9)	Oesch (1977)
Bacterial reverse mutation assay (Ames test)	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	Bentazone (83/3); 0, 20, 100, 500, 2500, 5000 μg/ml	96.7	Negative (±S9)	Engelhardt & Gelbke (1983)
Bacterial reverse mutation assay (Ames test and *E. coli* reverse mutation assay)	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538; *E. coli* WP2 uvrA	Bentazone; 0, 20, 100, 500, 2500, 5000 μg/ml	92.6	Negative (±S9)	Engelhardt & Gelbke (1985a)
Bacterial reverse mutation assay (Ames test)	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	Bentazone sodium (pure and technical grade); 0, 500, 1000, 2500, 5000, 7500, 10 000 μg/ml	Pure: 99.5 Technical grade: 47.7	Negative (±S9)	Engelhardt & Gelbke (1985b)
Bacterial reverse mutation assay (Ames test)	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538; *E. coli* WP2 hcr	Bentazone; up to 5000 μg/ml	Not given	Negative (−S9)	Moriya et al. (1983)
Bacterial reverse mutation assay (Ames test)	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538; *E. coli* WP2 hcr	Bentazone	99.9	Negative (±S9)	Jeang & Li (1978)
Bacterial reverse mutation assay (Ames test and *E. coli* reverse mutation assay)	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538; *E. coli* WP2 hcr	Bentazone	Not given	Negative (±S9)	Shirasu et al. (1982)
DNA damage and repair (SOS chromotest)	*E. coli*	Bentazone	Not given	Negative (±S9)	Xu & Schurr (1990)
DNA damage and repair (mitotic gene conversion assay)	*Saccharomyces cerevisiae* D4	Bentazone	Not given	Negative (−S9)	Siebert & Lemperle (1974)
DNA damage and repair (mitotic gene conversion assay)	*S. cerevisiae* D4	Bentazone	Not given	Negative (−S9)	Zimmermann et al. (1984)

Study	Strain/species	Substance; concentration/dose	Purity (%)	Result	Reference
Chromosomal aberration assay in mammalian cells	CHO cells	Bentazone 0, 500, 1000, 2000, 3000 μg/ml (−S9) 0, 2000, 3000, 4000, 5000 μg/ml (+S9)	Not given	Negative (±S9)	Taalman (1987)[a]
Forward mutation assay in mammalian cells (HPRT test)	CHO cells	Bentazone technical; 0, 100, 464, 1000, 2150, 4640, 10 000 μg/ml	93.9	Negative (−S9) Negative with rat S9 mix Weakly positive with mouse S9 mix	Jaeckh & Gelbke (1985)
Forward mutation assay in mammalian cells (HPRT test)	CHO cells	Bentazone technical (84/140); 1250, 2500, 5000, 7500, 10 000, 12 500, 15 000 μg/ml	93.9	Negative (−S9) Negative with rat or mouse S9 mix	den Boer (1985)[b]
Forward mutation assay in mammalian cells (HPRT test)	CHO cells	Bentazone technical; 0, 100, 600, 1200, 2500, 5000 μg/ml	97.6	Negative (±S9)	Muellerschoen (1991)[a]
In vivo					
Chromosome analysis (micronucleus test)	NMRI mouse	Bentazone technical; 0, 200, 400, 800 mg/kg bw; single application	95.6	Negative	Engelhardt & Gelbke (1985c)[b]
Chromosome analysis (micronucleus test)	Wistar rat	Bentazone technical; 0, 27.5, 55, 110, 220, 700 mg/kg bw; two oral administrations with 24 h time lag	Not given	Negative	Postica et al. (1982)
Unscheduled DNA synthesis	B6C3F1 mouse	Bentazone technical; 40–360 mg/kg bw; single application	Not given	Negative	Cifone (1985a)[b]
Mutation assay in germ cells (dominant lethal test)	Sprague-Dawley rat	Bentazone; dietary concentrations of 20, 60, 180 ppm over 13 weeks	Not given	Negative	Leuschner (1971)
Mutation assay in germ cells (dominant lethal test)	NMRI mouse	Bentazone technical; single intraperitoneal application of 195 mg/kg bw	Not given	Negative	Hofmann & Peh (1973)
Unscheduled DNA synthesis	B6C3F1 mouse primary hepatocytes	Bentazone; 0.05–1004 μg/ml in Williams' medium E	Not given	Negative	Cifone (1985b)

CHO, Chinese hamster ovary; DNA, deoxyribonucleic acid; S9, 9000 × *g* supernatant fraction of rat liver homogenate

[a] Complied with GLP.

[b] QA statement attached.

After the acclimatization period, F_0 parental animals continuously received the test substance throughout the entire study. At least 70 days after the beginning of treatment, male and female rats of the same dose groups were mated overnight. Females were allowed to deliver and rear their pups (F_1 generation pups) until day 4 or 21 after parturition. After weaning of the F_1 pups, the F_0 generation parental animals were sacrificed, and 25 male and 25 female F_1 pups of each treatment group were randomly selected as F_1 generation parental animals. All selected animals were treated with the test substance at the same dose level as their parents from post-weaning through adulthood up to about 1 day before they were sacrificed. At least 123 days after assignment of the F_1 generation parental animals, the males and females were paired one male to one female for a maximum of 19 days. No siblings were paired.

Like F_0 females, F_1 females were allowed to litter and rear their pups (F_2 generation pups) until day 4 (standardization) or day 21 after parturition. Shortly after weaning of the F_2 pups, the F_1 parental animals were sacrificed.

Males and females were paired at a 1:1 ratio for a maximum of 3 weeks. Vaginal smears were taken daily and examined for the presence of sperm and/or appearance of a vaginal plug. If evidence of mating was detected, pairing of the animals was discontinued. The day on which sperm were detected was denoted gestation day (GD) 0, and the following day, GD 1.

On postnatal day (PND) 4, the size of each litter was adjusted by eliminating extra pups by random selection to yield, as near as possible, four males and four females per litter. The surplus pups were sacrificed and examined macroscopically.

The average compound intakes for the females in the respective study periods are given in Table 14.

Table 14. Average bentazone intake in female parental rats

Group	Average bentazone intake (mg/kg bw per day)					
	200 ppm		800 ppm		3200 ppm	
	Average	Min/max	Average	Min/max	Average	Min/max
F_0 females (premating)	17.0	13/21	66.9	53/81	269	209/327
F_0 females (gestation)	14.7	13/16	60.7	53/67	247	226/264
F_0 females (lactation)	29.7	22/38	111	80/141	473	356/579
F_1 females (premating)	15.9	13/24	64.4	50/98	2261.6	209/372
F_1 females (gestation)	14.3	14/15	59.3	55/63	238.7	230/249
F_1 females (lactation)	29	22/36	121.3	91/152	492	357/590

From Suter et al. (1989)
max, maximum; min, minimum

The animals (i.e. parental animals and pups) were examined for mortality and evident signs of toxicity twice daily. Towards the end of the gestation period, females were examined twice daily for signs of parturition.

Females without litters and dams that lost their litters were killed together with the dams after weaning the pups and necropsied for the examination of the organs, including ovaries, uterus and uterine contents.

Body weight of parental animals was determined at weekly intervals with the exception of mating periods. The F_0 and F_1 generation parental females were weighed on the day of positive evidence of sperm (GD 0) and on GDs 7, 14 and 21, and females with litters were weighed on the day after parturition (PND 1) and on PNDs 4, 7, 14 and 21.

Pup body weights were determined on the day after birth (PND 1) and on PNDs 4 (before standardization), 7, 14 and 21. Feed consumption was recorded weekly for parental animals. On the day of birth (PND 0), the sex of the pups was determined.

All F_0 and F_1 adult animals selected for breeding were sacrificed when they were no longer necessary for assessment of reproductive effects. Excess F_1 and F_2 pups after standardization of litter sizes were sacrificed on day 4 postpartum, examined for possible defects and discarded. F_1 pups not selected for mating and all F_2 pups were sacrificed and examined for defects after weaning.

No treatment-related clinical signs of toxicity were observed. In the F_0 generation, there were a few transient signs in single animals of the control, 200 and 800 ppm groups. In the F_1 generation, signs were rare and mostly transient. In the 3200 ppm group, one female (#394) showed slight sedation and heavy body weight loss on day 4 postpartum. No treatment-related mortality was observed throughout the study.

Body weight development was impaired in high-dose parental F_0 and F_1 animals. For the males, test article–related decreased mean body weights were noted in the 3200 ppm group of the F_1 generation during both the prepairing and post-pairing periods. Similar mean body weight gain was noted in all groups of both generations during the post-pairing period.

For the females of the F_0 and F_1 generations, test article–related slightly decreased mean body weights (with statistical significance in both the F_0 and F_1 generations at isolated intervals) were noted in the 3200 ppm group during the prepairing, gestation and lactation periods. Mean body weight gain was slightly decreased during the prepairing period, similar during the gestation period and slightly increased during the lactation period when compared with the other treated groups (200 or 800 ppm) and the control group. Similar mean body weight gain and mean body weights were noted for the females of group 1 (control, 0 ppm), group 2 (200 ppm) and group 3 (800 ppm) at all periods for both generations. No test article–related changes in mean feed consumption were noted at any period for the F_0 or F_1 females of any dosed group when compared with the corresponding controls. The slight and, at some time points, statistically significant differences from the control values that were noted, mainly in the 3200 ppm group, but also in the other treated groups of the F_1 females, were considered to be of incidental origin. No clear dose–response relationship was evident.

Relative feed consumption corresponded to the feed consumption and body weights; no test article–related differences between the dose groups and the control group in either the F_0 or the F_1 generation were noted at any time point of any period.

As regards the effect on mating, gestation and rearing, for the parent females of both generations (F_0 and F_1), none of the parameters—numbers of females paired, mated, pregnant, bearing or rearing young, mating performance, fertility, duration of gestation and parturition or nursing ability—were impaired. The mean number of implantation sites, mean number of pups, postimplantation loss and breeding loss were similar in all groups.

Mean body weights of the pups were decreased and significantly different from controls during the lactation period in groups 3 (800 ppm) and 4 (3200 ppm) in both F_1 and F_2 generations. For the pups of the F_1 generation, decreased mean body weight was noted in groups 3 (800 ppm) and 4 (3200 ppm) from day 1 postpartum, but no clear dose–response relationship was evident.

In the F_2 generation, mean body weights for groups 3 (800 ppm) and 4 (3200 ppm) were similar to that of the control group on day 1 postpartum. Dose-dependent, slightly decreased body weight gain was noted thereafter during the lactation period.

The sex ratios of pups, the pup loss during the lactation period (before and after culling) and the physical development, health condition, viability and behaviour of the pups were similar in all dose groups up to the highest dose level of 3200 ppm, when compared with that of the control group.

No test article–related macroscopic changes were observed in either the F_0 or F_1 parent animals or the F_1 or F_2 pups of any group. The isolated findings described above were considered to be incidental in origin and not related to treatment with the test article.

In F_1 litters, no test article–related findings were evident in any dose group. No malformed or anomalous pups were noted in any group. Stray findings were observed in single cases in each group, which were considered to be incidental and not related to treatment with the test article.

In F_2 litters, no test article–related findings were noted in any dose group. Slight oedema in the upper region of the body, cheilognathoschisis, nose and nasal opening missing, breathing through mouth, microphthalmia or anophthalmia, encephalocele, missing tail, absence of external sex organs, and anal and uretal openings closed were observed at external examination.

No evidence of a teratogenic effect was observed in any group in either generation.

Based on the results of this two-generation reproductive toxicity study with bentazone technical in Wistar rats, the NOAEL for parental and offspring toxicity was considered to be 200 ppm (equal to about 14 mg/kg bw per day), based on the effects on body weight gain noted in F_1 generation males and females, reduced parental feed consumption and reduced pup body weight resulting from parental toxicity at 800 ppm (equal to about 59 mg/kg bw per day) in F_0 generation females that were dosed with the highest dietary concentration of 3200 ppm (equal to about 240 mg/kg bw per day) and in the F_1 and F_2 pups treated with 800 ppm and 3200 ppm, not at delivery, but during the lactation period.

Except for these findings, no interferences with the development and reproduction of the two generations were noted, and no teratogenic effects were observed up to the highest dose level of 3200 ppm (equal to about 238 mg/kg bw per day). A formal GLP compliance and QA statement was included in the report (laboratory certified by Eidgenössisches Departement des Innern, Bern, Switzerland) (Suter et al., 1989).

The herbicide bentazone was positively evaluated for inclusion in Annex I to Directive 91/414/EEC concerning the placing of plant protection products on the market based on the dossier submitted in 1995 by BASF AG. With regard to developmental end-points, bentazone has been evaluated in a two-generation reproductive toxicity study (Suter et al., 1989; see above). This study showed slightly reduced pup weight effects in the F_1 and F_2 generations at the middle dose (800 ppm) in the absence of obvious effects on parental weights, which were observed only at the high dose (3200 ppm). However, data from the long-term study of toxicity in rats (Takehara, 1984a) showed significant haematological and clinical chemistry alterations at 800 ppm.

Although the European Commission final review report on bentazone (European Commission, 2000) considered the effects demonstrated in the long-term study for the evaluation of parental toxicity, these effects were not considered in the most current evaluation of the same data by the USEPA (2010). Thus, the USEPA considers bentazone to affect offspring at non-parentally toxic doses.

In order to reassess these conflicting evaluations and to get a better picture about the origin of the pup weight effects, the two-generation reproductive toxicity study was re-evaluated. Due consideration was given to historical control data, and a focus on cofactors was included, including individual animal feed consumption and animal weight data as well as litter size distribution, in order to decide whether the pup weight impairment is a primary substance effect of bentazone or a secondary effect due to impairment of the dams.

Historical control data are valuable to differentiate between effects on a concurrent control group observed in a study and the inherent variability of biological parameters in studies conducted according to the same protocol in the same laboratory. The historical control data during the years 1985–1989 for this two-generation reproductive toxicity study with bentazone are compiled in a separate report (Gerspach, 2011) and are based on eight two-generation studies conducted in the same strain of rats.

The existence of an inverse relationship between pup body weight development and litter size, at least until culling at PND 4, due to competition for maternal milk is intensively described in Agnish & Keller (1997). This characteristic is also seen in the historical control data. Chahoud &

Paumgartten (2009) introduced an approach to standardize pup weight data for litter size effects by correction factors generated in a historical control cohort until the day of culling.

The reduced mean pup weight in the mid-dose F_1 generation was shown to be, to a considerable extent, due to the small litter size of the control group, as normalization of the mean pup weight to the litter size reduced this difference from a maximal 12% to 7%. In a next step, the remaining pup weight deviations were shown to be associated with litters from F_0 dams showing a significantly reduced feed intake and body weight gain in the relevant period of the lactation phase (PNDs 1–4). Although the group mean values of the middle dose do not vary significantly from the control group values, the individual analysis of the data showed that the litters with significantly reduced pup weights arise from dams showing relevant signs of toxicity, as demonstrated by a transient feed refusal within the early lactation phase and a severe maternal body weight loss or body weight gain reduction. Focusing only on those dams with clearly affected litters revealed reduced mean maternal feed consumption between PNDs 1 and 4 to 38% of the concurrent control and a mean body weight loss of 1.7 g between PNDs 1 and 4 in comparison with the control group, which gained about 16.7 g in this sensitive time frame. The respective pup body weight development is most likely impaired as a consequence of this nutritional deficit. A recovery was observed in dams and pups towards the end of the lactation phase, when the feed consumption levels were comparable with those of the control values. In the high-dose group, a maternal body weight reduction was apparent in all periods of the study, and the reduction in pup weight was shown to be attributable to a high litter size and/or a reduced maternal feed intake.

Similarly, the small mean litter size of the control animals in the F_2 generation led to higher mean pup weights in the control litters as compared with the treated groups. This led to an artificially high deviation of the treated animals from the control, but actually the F_2 generation mean pup weights are well within the naturally occurring variability of this parameter.

Therefore, this evaluation definitely supports the European conclusion that pup weight effects were observed only at maternally toxic levels. However, this re-evaluation showed the maternally toxic dose to lie at 800 ppm for the F_0 parental generation. The calculation of the effect levels in milligrams per kilogram body weight was done under consideration of the actual intake during the period where the toxicity was observed—namely, during the lactation period, PNDs 1–4.

The following relevant effect levels in parts per million and milligrams per kilogram body weight per day (according to the substance intake data) are considered appropriate for the two-generation study with bentazone. The NOAEL (maternal and developmental) is 200 ppm (equal to 22 mg/kg bw per day), based on the mean substance intake of F_0 dams between PNDs 1 and 4 during lactation, with a LOAEL (maternal and developmental) of 800 ppm (equal to 80 mg/kg bw per day). The resulting effect levels were derived on the basis of maternal toxicity evident for the F_0 females especially between PNDs 1 and 4 in the mid- and high-dose groups (800 and 3200 ppm) and in the F_1 females only in the high-dose group (3200 ppm). Reproductive/developmental toxicity is based on the slightly decreased pup body weights seen at PNDs 4 and 7 for the F_1 pups in the mid- and high-dose groups (800 and 3200 ppm) and for the F_2 pups in the high-dose group secondary to maternal toxicity (Chahoud & Paumgartten, 2009; Gerspach 2011; Kemény, 2011).

(b) Developmental toxicity

Rats

Bentazone (batch number and purity not given) was administered orally by gavage to groups of 20–36 pregnant Sprague-Dawley rats from day 6 to day 15 post-coitum. The doses administered in a 1% aqueous tylose solution were 0 (control), 22.2, 66.7 and 200 mg/kg bw per day. Two control groups were included. The rats were housed in pairs under controlled conditions and received standardized diet and water ad libitum. Clinical observations were recorded daily. Body weight was determined 3 times a week and on day 20 post-coitum. On this day, all animals were sacrificed and necropsied. The fetuses were dissected from the uterus, weighed, sexed and checked for any morphological abnormalities (external, gross pathological and skeletal examinations).

Following administration of the two lower doses, neither maternal toxicity nor embryo/fetal toxicity could be detected. In the highest dose group receiving 200 mg/kg bw per day, the resorption rate was drastically increased. In addition, the fetuses showed a decrease in body weight, an increase in the number of runts and an increase in the frequency of anasarca. The occurrence of anasarca was confined to this group. The total summary incidence of fetuses with anomalies of all types was also elevated. In contrast, maternal toxicity was not observed at this dose level.

In view of the above, the NOAEL was 200 mg/kg bw per day for maternal toxicity and 66.7 mg/kg bw per day for embryo/fetal toxicity, based on the high resorption rate and the fetal findings at the top dose level, which might suggest a fetotoxic or teratogenic potential of the test compound.

Although the study was conducted according to the "Guidelines for reproduction studies for safety evaluation of drugs from human use" (USFDA, 1966), it was not GLP compliant (Zeller & Peh, 1971).

The same study was repeated 6 years later. Bentazone (purity 92.5%) was tested for its prenatal toxicity in Sprague-Dawley rats. The test substance was administered at the same doses used in the previous study. Each group consisted of 26–29 rats. The animals were housed two per cage under controlled conditions and had free access to standardized diet and drinking-water. Clinical observations and mortality were checked daily. Body weight was determined 3 times a week, on day 0 of pregnancy and on days 6, 11, 15 and 20 post-coitum. On day 20, all animals were sacrificed and necropsied. The fetuses were dissected from the uterus, weighed, sexed and checked for any morphological abnormalities (external, gross pathological and skeletal examinations).

Bentazone was tolerated by all animals without any clinical symptoms and with no adverse effect on body weight or body weight gain. No animal died during the study period. No gross pathological changes were found. No differences between the control group and the substance-treated groups were noted with respect to conception rate, number of live or dead implantations or resorptions. Body weight of fetuses, their length and placenta weight remained unaffected. The examination of the fetuses did not reveal any abnormal findings.

Under the conditions of this study, no embryo/fetal toxicity or teratogenicity was noted. The NOAEL for both maternal and embryo/fetal toxicity was 200 mg/kg bw per day, the highest dose tested. The evidence of a fetotoxic or teratogenic potential of bentazone obtained in the previous study (see above) was not confirmed. Although the study was conducted according to the "Guidelines for reproduction studies for safety evaluation of drugs from human use" (USFDA, 1966), it was not GLP compliant (Hofmann & Merkle, 1978a).

In another study of embryotoxicity (including teratogenicity), bentazone (batch N 187, purity 97.8%) was administered daily to 25 presumably pregnant Wistar/HAN rats by stomach tube during GDs 6–15 at dose levels of 0, 40, 100 and 250 mg/kg bw per day. The age of the animals was at least 12 weeks, and the animals weighed around 185–225 g (post-coitum).

The treatment did not elicit any adverse effects at the low and middle doses. It did not produce any consistent signs of systemic maternal toxicity, such as clinical signs, mortality, changes in mean body weight or decreases in feed consumption. At the high dose, a slightly but significantly reduced maternal feed consumption was apparent (Table 15).

The maternal toxicity NOAEL was 250 mg/kg bw per day, the highest dose tested. Developmental toxicity, observed at 250 mg/kg bw per day, the highest dost tested, was characterized by an increase in post-implantation loss and a statistically significant increase in mean fetal resorptions per dam (but no increase in embryonic resorptions). This was accompanied by a statistically significant depression in the body weights (10.4%) of those fetuses surviving until day 21 sacrifice (Table 16).

Table 15. Feed consumption and body weight development in rats administered bentazone during days 6–15 of gestation

Parameter	0 mg/kg bw per day	40 mg/kg bw per day	100 mg/kg bw per day	250 mg/kg bw per day
Feed consumption (g/animal per day)				
Days 0–6	19.8	19.9	20.2	19.9
(% change relative to control)	—	(+0.5)	(+2.0)	(+0.5)
Days 6–11	21.3	21.1	20.6	20.1*
(% change relative to control)	—	(−0.9)	(−3.3)	(−5.6)
Days 11–16	22.5	21.9	22.1	21.8
(% change relative to control)	—	(−2.7)	(−1.8)	(−3.1)
Days 16–21	23.0	22.3	22.5	22.1
(% change relative to control)	—	(−3.0)	(−2.2)	(−3.9)
Body weight gain (g)				
Days 0–6	22	21	25	23
(% change relative to original weight at start of treatment)	(10.9)	(10.4)	(12.8)	(11.4)
Days 6–11	19	18	15	16
(% change relative to original weight at start of treatment)	(8.5)	(8.1)	(6.8)	(7.1)
Days 11–16	26	23	26	25
(% change relative to original weight at start of treatment)	(10.7)	(9.6)	(11.0)	(10.4)
Days 16–21	51	49	51	43
(% change relative to original weight at start of treatment)	(19.0)	(18.6)	(19.5)	(16.2)
Days 6–21	96	90	92	84
(% change relative to original weight at start of treatment)	(43.0)	(40.5)	(41.6)	(37.5)

From Becker et al. (1987a)
* $P < 0.05$

Bentazone also produced an effect upon the rate of growth, as evidenced by delayed or absence of ossification in the phalangeal nuclei of the extremities (digits of forelimb and hindlimb), sternebrae and cervical vertebrae. Examination of selected sites indicated that there was incomplete ossification of sternebra 5 (5 fetuses, 3 litters in controls versus 19 fetuses, 14 litters at the highest dose tested), absence of ossification in the metatarsals of toe 1 of the right hindlimb (18 fetuses, 8 litters in controls versus 53 fetuses, 19 litters at the highest dose tested) and absence of ossification in cervical vertebra 7 (1 fetus, 1 litter in control versus 9 fetuses, 5 litters at the highest dose tested) (Table 17).

The incidences of abnormal skeletal findings are shown in Table 18. The fetuses in group 4 (250 mg/kg bw per day) with incompletely ossified vertebrae and/or sternebrae are considered to mirror a delayed maturation, indicated by the reduced body weights of fetuses, and not a specific effect of the test article on skeletal development.

Table 16. Caesarean section data

Parameter	0 mg/kg bw per day	40 mg/kg bw per day	100 mg/kg bw per day	250 mg/kg bw per day
Pregnancy status				
Mated (*n*)	25	25	25	25
Pregnant (*n*)	24	22	24	25
Conception rate (%)	96	88	96	100
Aborted (*n*)	0	0	0	0
Dams with viable fetuses (*n*)	24	22	24	25
Dams with all resorptions (*n*)	0	0	0	0
Mortality (*n*)	0	0	0	0
Pregnant at terminal sacrifice (*n*)	24	22	24	25
Caesarean section data				
Corpora lutea				
- mean (*n*)	13.5 ± 1.8[a]	13.5 ± 1.7	14.0 ± 1.7	13.7 ± 1.6
- total number (*n*)	324	297	335	343
Implantation sites				
- mean (*n*)	11.8 ± 2.2	11.5 ± 3.2	12.4 ± 2.2	12.2 ± 2.2
- total number (*n*)	282	253	298	305
Preimplantation loss (*n*)	42	44	37	38
Preimplantation loss (%)	13.0	14.8	11.0	11.1
Preimplantation loss mean (*n*)	1.8	2.0	1.5	1.5
Resorptions				
- mean/dam (*n*)	0.9	1.0	1.1	2.7
- total number (*n*)	21	21	26	67
- % of implantations	7.4	8.3	8.7	22.0
Early resorptions				
- mean/dam (*n*)	0.9	1.0	1.0	0.9
- total number (*n*)	21	21	25	23
- % of implantations	7.4	8.3	8.4	7.5
Late resorptions				
- mean/dam (*n*)	0.0	0.0	0.0	1.8*
- total number (*n*)	0	0	1	44*
- % of implantations	0	0	0.3	14.4*
Dead fetuses (*n*)	0	0	0	0
Live fetuses				
- mean/dam (*n*)	10.9 ± 2.1	10.5 ± 3.1	11.3 ± 2.1	9.5 ± 4.1
- total number (*n*)	261	232	272	238
- % of implantations	92.6	91.7	91.3	78.0
Total live female fetuses				
- mean/dam (*n*)	5.4 ± 2.0	5.8 ± 2.3	5.6 ± 1.8	5.3 ± 2.7
- total number (*n*)	129	127	134	133
- mean (%)	49.9	54.7	49.3	55.9
Total live male fetuses				

Parameter	0 mg/kg bw per day	40 mg/kg bw per day	100 mg/kg bw per day	250 mg/kg bw per day
- mean/dam (*n*)	5.5 ± 1.7	4.8 ± 1.9	5.8 ± 1.8	4.2 ± 2.0
- total number (*n*)	132	105	138	105
- mean (%)	50.6	45.3	50.7	44.1
Mean fetal weight (g)				
- males	4.9 ± 0.2	5.1 ± 0.3	5.0 ± 0.3	4.4 ± 0.4
- females	4.7 ± 0.3	4.7 ± 0.3	4.7 ± 0.3	4.1 ± 0.4
- males and females	4.8 ± 0.2	4.9 ± 0.3	4.9 ± 0.3	4.2 ± 0.4*

From Becker et al. (1987a)
* $P \leq 0.05$
[a] Standard deviation.

Table 17. Skeletal investigations of fetuses without abnormal findings

	Group 1 0 mg/kg bw per day	Group 2 40 mg/kg bw per day	Group 3 100 mg/kg bw per day	Group 4 250 mg/kg bw per day
No. of skeletons investigated	*132*	*115*	*143*	*120*
Sternebrae				
(B) sternebra 1	—	—	—	1 (0.8)
(B) sternebra 2	1 (0.8)[a]	—	—	1 (0.8)
(B) sternebra 3	—	—	—	1 (0.8)
(B) sternebra 4	—	—	—	1 (0.8)
(B) sternebra 5	5 (3.8)	3 (2.6)	10 (7.0)	18 (15.0)
Cervical vertebrae				
(A) vertebra 1	21 (15.9)	14 (12.2)	15 (10.5)	47 (39.2)
(C) vertebra 1	6 (4.5)	5 (4.3)	7 (4.9)	5 (4.2)
(A) vertebra 2	33 (25.0)	23 (20.0)	25 (17.5)	59 (49.2)
(A) vertebra 3	11 (8.3)	1 (0.9)	3 (2.1)	27 (22.5)
(A) vertebra 4	6 (4.5)	—	1 (0.7)	15 (12.5)
(A) vertebra 5	4 (3.0)	—	—	15 (12.5)
(A) vertebra 6	3 (2.3)	—	—	11 (9.2)
(A) vertebra 7	1 (0.8)	—	—	9 (7.5)
Ribs				
(D) rib no. 14 left side	1 (0.8)	1 (0.9)	3 (2.1)	5 (4.2)
(D) rib no. 14 right side	2 (1.5)	—	2 (1.4)	5 (4.2)
Phalangeal nuclei[b]				
Left-hand limb				
(A) toe 1 distal phalanx	25 (18.9)	19 (16.5)	20 (14.0)	27 (22.5)
(A) toe 1 metatarsalia	15 (11.4)	17 (14.8)	17 (11.9)	49 (40.8)
(A) toe 2 distal phalanx	—	—	—	3 (2.5)
(A) toe 2 proximal phalanx	89 (67.4)	73 (63.5)	88 (61.5)	110 (91.7)
(A) toe 3 distal phalanx	—	—	—	3 (2.5)
(A) toe 3 proximal phalanx	61 (46.2)	51 (44.3)	53 (37.1)	95 (79.2)
(A) toe 4 distal phalanx	—	—	—	3 (2.5)
(A) toe 4 proximal phalanx	58 (43.9)	45 (39.1)	48 (33.6)	93 (77.5)
(A) toe 5 distal phalanx	12 (9.1)	5 (4.3)	12 (8.4)	10 (8.3)
(A) toe 5 proximal phalanx	117 (88.6)	97 (84.3)	130 (90.9)	118 (98.3)

Table 17 (continued)

	Group 1 0 mg/kg bw per day	Group 2 40 mg/kg bw per day	Group 3 100 mg/kg bw per day	Group 4 250 mg/kg bw per day
Right-hand limb				
(A) toe 1 distal phalanx	18 (13.6)	11 (9.6)	15 (10.5)	19 (15.8)
(A) toe 1 metatarsalia	15 (11.4)	17 (14.8)	17 (11.9)	53 (44.2)
(A) toe 2 distal phalanx	—	—	—	3 (2.5)
(A) toe 2 proximal phalanx	89 (67.4)	78 (67.8)	92 (64.3)	114 (95.0)
(A) toe 3 distal phalanx	—	—	—	3 (2.5)
(A) toe 3 proximal phalanx	62 (47.0)	55 (47.8)	57 (39.9)	103 (85.8)
(A) toe 4 distal phalanx	—	—	—	3 (2.5)
(A) toe 4 proximal phalanx	58 (43.9)	53 (46.1)	52 (36.4)	99 (82.5)
(A) toe 5 distal phalanx	10 (7.6)	5 (4.3)	8 (5.6)	10 (8.3)
(A) toe 5 proximal phalanx	118 (89.4)	99 (86.1)	125 (87.4)	120 (100.0)
Calcaneum				
(A) left side	117 (88.6)	87 (75.7)	115 (80.4)	112 (93.3)
(A) right side	115 (87.1)	88 (76.5)	114 (79.7)	110 (91.7)

From Becker et al. (1987a)

A, ossification still absent; B, incompletely ossified; C, ossification centre dumbbell shape; D, supernumerary rib

[a] Percentage of skeletons showing the finding.

[b] Medical phalangeal nuclei of all fetuses still absent.

Table 18. Incidence of abnormal skeletal findings

Parameter	0 mg/kg bw per day	40 mg/kg bw per day	100 mg/kg bw per day	250 mg/kg bw per day
Litters evaluated (*n*)	25	22	24	25
Fetuses evaluated (*n*)	132	115	143	120
Live fetuses (*n*)	132	115	143	120
Dead fetuses (*n*)	0	0	0	0
Total abnormal skeletal findings				
Fetal incidence (%)	0 (0.0)	0 (0.0)	1 (0.7)	9 (7.5)
Litter incidence (%)	0 (0.0)	0 (0.0)	1 (4.2)	7 (28)
Individual abnormal skeletal findings, no. (corresponding mean fetal body weight in grams)				
Non-ossified sternebra				
- No. 2	0	0	1 (4.4)	0
- No. 6	0	0	0	4 (3.2)
- Nos 2, 5 and 6	0	0	0	1 (2.8)
Abnormally ossified and bipartite sternebra no. 4, abnormally ossified sternebra no. 5	0	0	0	1 (4.5)
Non-ossified vertebral body no. 1, non-ossified sternebrae nos 5 and 6	0	0	0	1 (2.6)
Non-ossified vertebral body no. 1	0	0	0	2 (3.4)

From Becker et al. (1987a)

The developmental NOAEL was 100 mg/kg bw per day, based on increased post-implantation loss, skeletal variations (incomplete or absent ossification in the phalangeal nuclei of the extremities, sternebrae and cervical vertebrae) and reduced body weights of fetuses surviving to day 21 at 250 mg/kg bw per day. There were no indications of teratogenic potential in this study up to the highest dose level tested. The study is GLP compliant (Becker et al., 1987a).

Bentazone (batch N 169, purity 93.9%) was administered daily to presumably pregnant Charles River CD rats via the diet during the entire gestation period (days 0–21) at a concentration of 0, 2000, 4000 or 8000 ppm (calculated to be equal to 0, 162, 324 and 631 mg/kg bw per day, respectively). The age of the animals was 7 weeks at the time of purchase. The animals were acclimatized for 11 days before administration of the test substance. The group mean weight was between 216 and 217 g.

No treatment-related effects were observed in the group dosed with 2000 ppm. The dietary level of 4000 ppm caused significant increases in water consumption and amniotic fluid weight in pregnant rats. Pregnant animals dosed with 8000 ppm displayed decreased feed intake, increased water consumption, suppression of body weight gain and various clinical signs suggesting haemorrhagic diathesis and signs of disturbance of fetal growth, characterized by an increased incidence of reduced fetal body weight and reduced ossification of cervical vertebrae. Additionally, haemorrhages were found in the liver of some high-dose pups. These effects are interpreted as secondary manifestations of toxic effects on the pregnant rats rather than being a direct influence of the test substance on the fetus. The study did not reveal any teratogenic potential.

The NOAEL for maternal effects was 2000 ppm (equal to 162 mg/kg bw per day), on the basis of increased water consumption at 4000 ppm (equal to 324 mg/kg bw per day), and the developmental toxicity NOAEL was 4000 ppm (equal to 324 mg/kg bw per day), on the basis of decreased fetal weight gain and reduced ossification of cervical vertebrae at 8000 ppm (equal to 531 mg/kg bw per day). Although the study was conducted prior to the implementation of GLP, it is stated that the study was run according to the principles of GLP. A formal GLP compliance and QA statement was included in the report (Itabashi et al., 1983).

A published literature study on the teratological effects of the pesticide Basagran on embryos of the albino rat was submitted. Groups of three pregnant albino rats (strain and source not specified) were orally administered single doses of 0, 25, 90 or 200 mg of the formulation Basagran (origin and purity not submitted) per kilogram body weight (corresponding to bentazone doses of 0, 12.0, 43.2 and 96 mg/kg bw) by gavage on GD 6, 8, 11, 14 or 16. On GD 20, all animals were sacrificed and necropsied. The fetuses were dissected from the uterus. Resorptions were counted, and the skeletons of fetuses were examined. The fetal findings observed consisted of an increased resorption rate, retardation of fetal development, incomplete ossification and absence of some bones. The increased resorption rates were noted at comparable incidences in all treated groups, irrespective of the dose administered. The incidence and severity of the findings decreased with the later times of administration. Thus, the findings were time dependent, but not dose dependent. The publication gave no details on maternal toxicity. The results of the gross pathological examination of the fetuses were only summarized in this study, and the frequency of the changes was not reported. In addition, the results of the examination of the control animals were not given.

Because of the inconsistency of the data reported, this investigation is considered unacceptable for evaluation purposes. However, it provides supplementary information, as the time dependence of fetal effects was investigated. The study is not GLP compliant (El-Mahdi & Lotfi, 1988).

Rabbits

In a study of developmental toxicity, groups of 15 Himalayan rabbits (ChBB:HM) were given bentazone (purity 92.5%) at a dose of 50, 100 or 150 mg/kg bw per day by gavage on GDs 6–18. Two

additional groups served as untreated controls or vehicle controls. At the time of procurement, the rabbits were between 22 and 83 weeks old with a mean weight of 2.449 kg. After a 10-day acclimatization period, the rabbits were fertilized by artificial insemination. The animals were observed daily for clinical signs and for mortality. Body weights, body weight gains and feed consumption were measured each day. At necropsy, the uterus was removed and the animals were examined for gross pathology. The number of corpora lutea, conception rate, number of implantations (live and dead implantations and early, intermediate and late resorptions) and number of dead fetuses were determined. The fetuses were removed from the uterus by caesarean section and examined. The weight and length of the fetuses were measured, and the placentas were weighed. The heads of the fetuses were fixed, and transverse sections were made and examined; skeletal assessment was undertaken by radiological examination.

One dam in the untreated control group aborted. One death was seen in each of the groups receiving bentazone at 100 or 150 mg/kg bw per day (these dams had severe vaginal haemorrhages), and a dam in the group at 100 mg/kg bw per day bore six fetuses prematurely on day 26 after conception. A dam at 100 mg/kg bw per day aborted on day 26 or 27 post-coitum. No other adverse clinical signs were noted, and there was no test material–related effect on maternal body weight gain. Feed consumption was lower in the dosed groups and in the vehicle control groups than in the group of untreated controls. No test material–related macroscopic abnormalities were seen in the animals that were killed at study termination. No intergroup differences were seen in conception rate or numbers of implantations and corpora lutea. Fetal body weights were increased at 100 and 150 mg/kg bw per day; however, this is not likely to be an adverse effect. Fetal length and placental weight were not affected by treatment. There were no differences between the groups in the frequency of anomalies, variations and retardations observed.

Accordingly, the NOAEL for maternal and fetal toxicity for bentazone was 150 mg/kg bw per day, the highest dose tested. Bentazone was not teratogenic. The study is not GLP compliant (Hofmann & Merkle, 1978b).

Bentazone (batch N 187, purity 97.8%) was administered daily to presumably pregnant Chinchilla rabbits by stomach tube during GDs 6–18 at a dose level of 0, 75, 150 or 375 mg/kg bw per day. The age of the animals at pairing was between 4 and 7 months, with weight (post-coitum) 2513–3539 g. The animals were examined for mortality, signs and symptoms twice daily. All animals were weighed daily from day 0 to day 28 post-coitum. The body weight change of the animals was calculated from these results during the treatment period beginning at day 6 (immediately prior to the first administration) and ended on day 19 post-coitum (approximately 24 hours after the last administration). In addition, the body weight gain corrected for uterine weight at necropsy was calculated.

On day 28 post-coitum, all females were killed by cervical dislocation and the fetuses removed by caesarean section. Postmortem examinations, including gross macroscopic examination of all internal organs, with emphasis upon the uterus, uterine contents, position of fetuses in the uterus and number of corpora lutea, were performed and the results recorded. The uteri (and contents) of all pregnant females were weighed on the scheduled day of necropsy and used to determine the corrected body weight gain. The uteri of all females that were found at necropsy to be not pregnant were placed in an aqueous solution of ammonium sulfide (Salewski, 1964) to accentuate possible haemorrhagic areas of implantation sites. All tissues and organs of the females were discarded. The fetuses were removed from the uterus, weighed, examined for gross external abnormalities and prepared for internal examinations. All fetuses were dissected carefully, the body cavities (thorax, abdomen, pelvis) and the organs investigated and any abnormal findings recorded. The sex of each fetus was noted and recorded. The heads of all fetuses were fixed in a solution of trichloroacetic acid and formaldehyde. The heads were cross-sectioned, and the cephalic viscera were examined (Wilson & Warkany, 1965). Descriptions of any abnormalities were recorded. After evaluation, the individual sections were preserved in a solution of ethyl alcohol and glycerine (one head per container). The crania of all fetuses were examined for ossification after the scalps were removed. The trunks of all

fetuses were placed in a solution of potassium hydroxide for clearing and stained with alizarin red (modified technique; Dawson, 1926). The skeletons were examined, and all abnormalities and variations were recorded. The specimens were preserved individually in plastic bags. The stained trunks of fetuses and the sections of the heads of fetuses were preserved.

No mortality was observed in this study. No signs or symptoms were observed in any female of the control, low-dose and mid-dose groups. Treatment-related effects were limited to the high dose (375 mg/kg bw per day) and consisted of signs of abortion in one dam, indicated by five aborted placentas found on day 22 post-coitum (in this dam, a total post-implantation loss was ascertained during necropsy on day 28 post-coitum), and a slight reduction of the mean feed consumption during the treatment period. No test article–related differences in comparison with the vehicle control group data were noted in the remaining parameters recorded and in all data of the low-dose group (75 mg/kg bw per day) and the mid-dose group (150 mg/kg bw per day).

Besides this, a single incidental finding (hydrocephalus internus) in one fetus of the mid-dose group (150 mg/kg bw per day) was noted during the gross pathological investigations. During the skeletal investigations, isolated abnormal findings were noted in all groups, including the controls. There were no signs of a test article relationship (Table 19).

There were no indications of a teratogenic potential at any dose. Based on these results, the NOAEL for maternal and prenatal developmental toxicity in rabbits is 150 mg/kg bw per day, based on signs of abortion and reduction of maternal feed consumption at 375 mg/kg bw per day. The study was GLP compliant, and a QA statement was attached (Becker et al., 1987b).

(c) Effects on spermatogenesis

A published literature study on the effects of a low dose of bentazone on spermatogenesis in mice exposed during fetal, postnatal and adult life, which was made available to the Committee, was reviewed. In this paper, the potential reproductive hazard to humans resulting from exposure to bentazone in drinking-water was studied in mice. Bentazone was administered in drinking-water at a concentration of 30 µg/l to (a) 10 adult male mice (3 months old) for 100 days, resulting in a dose of 21 µg/kg bw per day, and (b) 12 male mice exposed in utero, during lactation (from three dams, each was allowed to nurse four male offspring) and up to PND 100, resulting in a dose of 14 µg/kg bw per day.

With regard to male reproductive parameters, no histopathological changes were seen. Sperm number and morphology were not affected by the treatment. There were also no changes when using synaptonemal complex immunostaining or when using the micronucleus and comet assays (measures of chromosomal damage). The only statistically significant effect seen was an alteration of the frequency of some stages of sperm maturation in both experimental groups compared with the concurrent control groups, with no consistent pattern. According to a review article by Creasy (1997), quantifying stage frequency is not appropriate as an end-point itself. This parameter is used only to identify cell loss in the case of spermatid retention.

Therefore, this study is considered as supplementary information that confirms the absence of male reproductive toxicity in mice at doses below those chosen for risk assessment. This evaluation is supported by USEPA (2010). The study is not GLP compliant (Garagna et al., 2005).

2.6 Special studies

(a) Neurotoxicity

In a subchronic neurotoxicity study in Wistar CrlGlxBrlHan:Wi rats, bentazone (batch N 187; purity 96.9%) was administered to groups of 10 rats of each sex per dose at a dietary concentration of 0, 300, 1000 or 3500 ppm (equal to 0, 21.9, 73.6 and 258.1 mg/kg bw per day for males and 0, 27, 86.4 and 306.3 mg/kg bw per day for females, respectively) for at least 91 days. Each group was subdivided into two subsets (A and B) in order to balance the groups for functional observational battery and motor activity measurements. The animals were assigned to the treatment groups by

means of computer-generated randomization lists based on body weights. All the parameters, including functional observational battery, were examined as per approved guidelines.

Table 19. Caesarean section data in rabbits[a]

	0 mg/kg bw per day	75 mg/kg bw per day	150 mg/kg bw per day	375 mg/kg bw per day
Pregnancy status				
Mated (*n*)	16	16	16	16
Pregnant (*n*)	16	16	16	15
Conception rate (%)	100	100	100	94
Aborted/resorbed (*n*)	0	0	0	1
Dams with viable fetuses (*n*)	16	16	16	14
Mortality (%)	0	0	0	0
Pregnant terminal sacrifice (*n*)	16	16	16	14
Cesarean section data				
Corpora lutea				
- mean/dam	7.8 ± 1.6[b]	7.7 ± 1.5	8.6 ± 1.1	8.9 ± 1.5
- total number (*n*)	125	123	137	124
Implantation sites				
- mean/dam	7.7 ± 1.9	7.4 ± 1.5	8.4 ± 1.1	8.4 ± 1.9
- total number (*n*)	123	119	134	118
Preimplantation loss (%)	1.6	3.3	2.2	4.8
Post-implantation loss (%)	4.1	4.2	3.7	3.4 (8.8[c])
Resorptions				
- mean/dam	0.3	0.3	0.3	0.3
- total number (*n*)	5	5	5	4
- % of implantations	4.1	4.2	3.7	3.4
Early resorptions				
- mean/dam	0.3	0.1	0.3	0.1
- total number (*n*)	4	2	4	1
- % of implantations	3.3	1.7	3.0	0.8
Late resorptions				
- mean/dam	0.1	0.2	0.1	0.2
- total number (*n*)	1	3	1	3
- % of implantations	0.8	2.5	0.7	2.5
Dead fetuses (*n*)	0	0	0	0
Live fetuses				
- mean/dam	7.4 ± 2.2	7.1 ± 1.6	8.1 ± 1.5	8.1 ± 1.9
- total number (*n*)	118	114	129	114
Total live female fetuses				
- total number (*n*)	56	56	75	56
- mean (%)	47.5	49.1	58.1	49.1
Total live male fetuses				
- total number (*n*)	62	58	54	58

	0 mg/kg bw per day	75 mg/kg bw per day	150 mg/kg bw per day	375 mg/kg bw per day
- mean (%)	52.5	50.9	41.9	50.9
Mean fetal weight (g)				
- males[d]	37.9 ± 3.8	38.5 ± 4.6	37.0 ± 2.6	36.0 ± 3.5
- females[d]	37.1 ± 4.5	37.6 ± 5.2	35.7 ± 2.3	35.4 ± 2.9
- males and females[d]	37.7 ± 3.9	38.0 ± 4.7	36.4 ± 2.1	35.7 ± 2.8
- males and females	36.8 ± 5.0	37.3 ± 5.7	36.1 ± 3.8	35.3 ± 4.5

From Becker et al. (1987b)
[a] This table excludes dams #53 and #64 of the high-dose group.
[b] Standard deviation.
[c] Post-implantation loss under consideration of dam #64 (125 implantations and 11 losses).
[d] Unweighted mean of litter means and variation between litters.

No treatment-related clinical signs were observed throughout the study. Incidental observations included alopecia at various regions of the body in one mid-dose (1000 ppm) male and two high-dose (3500 ppm) females; piloerection in one high-dose female; and injury to the left ear in one control female.

No mortality or any treatment-related ophthalmoscopic findings were observed throughout the study. However, at termination, the only findings consisted of corneal stipplings in five control and three high-dose males and two control and five high-dose females. The incidence of this finding was within the expected range for rats of this age.

No treatment-related effects on body weight or body weight gain were observed. In the absence of statistical significance, the slightly lower terminal body weight and lower overall body weight gain of high-dose males were considered to be incidental. A statistically significant decrease in mean daily feed consumption was observed in high-dose males at days 63 and 77. The isolated occurrence was not indicative of a relationship to treatment. Except for a decrease in low-dose males at day 49, no statistically significant differences in feed efficiency were noted in any treated group. The isolated occurrence and the lack of a dose–response relationship indicated that the statistically significant difference in low-dose males was incidental.

No treatment-related functional observational findings were observed at any dose level. No statistically significant differences in defecation, number of rearings, forelimb and hindlimb grip strength or foot splay width were observed in any treated group. During home cage observation, deviations from (rank) “zero values” were obtained in several animals. However, all findings were equally distributed between treated groups and controls. Parameters investigated included posture, tremors, convulsions, abnormal movements and impairment of gait. During open-field observations and sensory motor tests/reflexes, deviations from “zero values” were obtained in several animals. However, as all findings were equally distributed between treated groups and controls, were without a dose–response relationship or occurred in single animals only, these observations were considered incidental. No treatment-related changes in motor activity were noted in treated groups.

There were some statistically significant differences between control and treated groups; however, these changes were neither dose related nor consistent over time. Therefore, these changes were considered incidental. These changes consisted of (in low-dose males) increased overall activity at day 50, decreased activity at interval 4 on day −7 and increased activity in low-dose males at intervals 8–10 and at day 50; (in mid-dose males) increased activity at interval 2 on day −7, decreased activity at interval 10 on day 22 and increased activity at intervals 9 and 10 on day 50; (in high-dose males) increased activity at interval 2 on day −7 and decreased activity at intervals 7 and 10 on day 22; (in low-dose females) increased activity at intervals 2 and 3 on day 22 and increased activity at interval 2 on day 50; (in mid-dose females) increased activity at intervals 2 and 3 on day 22; and (in high-dose females) increased activity at interval 2 on day 22.

Terminal body weights were comparable between all groups. Likewise, no statistically significant differences in absolute and relative brain weights were observed. No macroscopic lesions were observed at necropsy. With the exception of axonal degeneration of lumbar ganglia in one control male, no neurohistopathological lesions were noted in this study.

Dietary administration of bentazone to rats at dose levels of 0, 300, 1000 and 3500 ppm did not result in any indication of neurotoxicity. Under the conditions of the present study, the NOAEL for neurotoxicity was 3500 ppm (equal to 258.1 mg/kg bw per day in males and 306.3 mg/kg bw per day in females), the highest dose tested. The study was GLP compliant, and a QA statement was attached (Kaspers et al., 2004).

(b) Studies on metabolites

6-Hydroxybentazone and 8-hydroxybentazone are major plant metabolites of bentazone. Because crops of treated plants can be consumed by humans, farm animals or pets, an exposure to both of these compounds might be expected in principle. Although both metabolites have been demonstrated to be formed in mammals and therefore can be regarded as included in toxicological testing of the parent compound, specific toxicological studies were performed.

It has been shown that the 8-hydroxy and 6-hydroxy metabolites of bentazone are of comparable toxicity by the oral route of administration and are both less toxic than the parent compound. Additionally, both metabolites were negative in the Ames assay for the potential to induce point mutations in bacteria. As it is unlikely that a hydroxy group shift in the bentazone ring system dramatically changes the toxicity, it was decided to perform further investigations on 8-hydroxybentazone as a reference substance.

Therefore, 8-hydroxybentazone was investigated in a subchronic feeding study, in several mutagenicity studies and in a prenatal developmental study. These investigations revealed that the metabolites have no mutagenic or teratogenic potential and are less toxic than the parent substance.

Acute toxicity

The acute toxicity of 6-hydroxybentazone and 8-hydroxybentazone is summarized in Table 20.

Table 20. Acute toxicity of bentazone metabolites

Species	Strain	Sex	Route	Batch no./ purity	LD_{50} (mg/kg bw)	Reference
6-Hydroxybentazone						
Rat	Wistar	Male Female	Oral	E-106251 / > 98%	> 5000	Kieczka & Kirsch (1987b)
Mouse	NMRI	Male Female	Oral	E-106251 / > 98%	> 5000	Kieczka & Kirsch (1987c)
8-Hydroxybentazone						
Rat	Wistar	Male Female	Oral	Batch not given / > 98.5%	> 5000	Kieczka & Kirsch (1987a)
Mouse	NMRI	Male Female	Oral	Batch not given / > 98.5%	> 5000	Kirsch & Hildebrand (1987)

Genotoxicity

The genotoxicity of 6-hydroxybentazone and 8-hydroxybentazone is summarized in Table 22.

Table 22. Genotoxicity of bentazone metabolites

Study	Test system	Concentration/ dose	Purity (%)	Results	Reference
6-Hydroxybentazone					
Bacterial reverse mutation assay (Ames test)	*S. typhimurium* TA98, TA100, TA1535, TA1537	20, 100, 500, 2500, 5000 µg/plate	> 98	Negative (±S9)	Engelhardt & Gelbke (1987b)
8-Hydroxybentazone					
Bacterial reverse mutation assay (Ames test)	*S. typhimurium* TA98, TA100, TA1535, TA1537, plate incorporation and preincubation assay; with/without S9 mix	20, 100, 500, 2500, 5000 µg/plate	99.9	Negative (±S9)	Engelhardt & Gelbke (1987a)
In vitro forward mutation assay in mammalian cells (HPRT test)	V79 cells; with/without S9 mix	Among others, 300, 1000, 2000, 3000; and 500, 1000, 2500, 5000 µg/ml	99.9	Negative (±S9)	Muellerschoen (1992)
In vivo mouse micronucleus test	NMRI mouse, male and female (single oral gavage; vehicle: 0.5% aqueous carboxymethylcellulose)	625, 1250, 2500 mg/kg bw	99.9	Negative	Engelhardt & Hoffmann (1993)

S9, 9000 × *g* supernatant fraction of rat liver homogenate

Subchronic oral toxicity (8-hydroxybentazone)

To study and determine the toxicity profile and NOAEL, 8-hydroxybentazone (batch no. L 47-213; purity 99.9%) was administered to groups of 10 rats (Chbb:Thom(SPF)) of each sex per dose group (42 days old and weighing around 186 [176–201] g [males] and 143 [134–150] g [females]) at a dietary concentration of 0, 400 (low dose), 1200 (intermediate dose) or 3600 ppm (top dose) (equal to 0, 28, 85 and 259 mg/kg bw per day for males and 0, 34, 101 and 304 mg/kg bw per day for females, respectively) for at least 90 days. The mean substance intakes for both sexes were 0, 31, 93 and 282 mg/kg bw per day, respectively. The animals were examined for all parameters as per guidelines.

The administration of 8-hydroxybentazone did not result in any substance-related effects. One low-dose male died on study day 27, and one mid-dose male died on study day 63. The cause of death could not be determined. These mortalities are not considered treatment related. No treatment-related ophthalmological findings were noted. The only findings observed at the end of the treatment period were mainly remainders of the pupillary membrane and occasionally corneal stippling. The body weight development, body weight changes and feed consumption of the treated male and female animals did not differ substantially from those of the control animals.

In terms of haematological parameters, the only statistically significant change seen was a slight increase in reticulocyte count in females at the end of the study, a finding not considered biologically relevant. No substance-related effects were noted in the clotting analyses. A few spurious statistically significant clinical chemistry findings were observed.

A few spurious statistically significant decreased concentrations of inorganic phosphorus at 1200 and 3600 ppm, minimally decreased concentrations of calcium at 1200 ppm and decreased concentrations of total bilirubin, total protein and globulins at 400 ppm were seen only in males and only at the end of the study. In the absence of a dose–effect relationship or associated adverse findings, these findings are not considered to be of toxicological relevance.

No treatment-related changes in urinary parameters were observed.

The only finding of statistical significance was a decrease in the absolute weight of the adrenal glands in males of the 400 ppm group compared with controls. There was no dose–response relationship, and there were no significant differences in the absolute weights of the adrenal glands at higher doses. Furthermore, the relative adrenal weights did not show significant differences when compared with the controls. Therefore, a substance-related effect can be ruled out. No other statistically significant changes in absolute or relative organ weights were observed.

All gross lesions and histopathological findings were biologically equally distributed over the control and treatment groups and are considered as having developed spontaneously.

Dietary administration of 8-hydroxybentazone to rats at dose levels up to 3600 ppm did not result in any compound-related effects. The NOAEL under the conditions of the present comparative study was 3600 ppm (equal to about 259 mg/kg bw per day in males and 304 mg/kg bw per day in females, or 282 mg/kg bw per day for both sexes combined), the highest dose tested. Therefore, the metabolite 8-hydroxybentazone proved to be less toxic than the parent compound bentazone. The study was GLP compliant, and a QA statement was attached (Mellert & Hildebrand, 1993).

Developmental toxicity of 8-hydroxybentazone

8-Hydroxybentazone was tested for its toxicity in Wistar (Chbb:THOM(SPF)) rats (77–79 days old, weighing 232.5 g [mean]). The test substance (batch no. 108 746, purity 99.9%) was administered as an aqueous suspension to 20–25 pregnant female rats per group by stomach tube at a dose of 40, 100 or 250 mg/kg bw on day 6 through day 15 post-coitum. A standard dose volume of 10 ml/kg bw was used. The control group was dosed with the vehicle only (0.5% aqueous carboxymethylcellulose solution). On day 20 post-coitum, all females were sacrificed and assessed by gross pathology. The fetuses were dissected from the uterus, sexed, weighed and further investigated for any external and/or skeletal findings.

There were no substantial substance-related effects on the dams concerning feed consumption, body weight, body weight change, uterine weights, corrected body weight change, or clinical and necropsy observations up to and including the dose of 250 mg/kg bw per day. There were no differences of biological relevance between the control and the substance-treated groups (40, 100 and 250 mg/kg bw per day) in conception rate, mean numbers of corpora lutea, total implantations, resorptions and live fetuses, fetal sex ratio or preimplantation and post-implantation losses.

No dose- or substance-related differences were recorded for placental and fetal body weights. The external, soft tissue and skeletal examinations of the fetuses revealed no differences between the control and the substance-treated groups that might be related to the test substance administration. Number and type of the fetal external, soft tissue and skeletal findings, which were classified as malformations, variations and/or retardations, recorded for the 40, 100 and 250 mg/kg bw per day fetuses were substantially similar to actual and/or historical control values.

Thus, under the conditions of this study, 8-hydroxybentazone caused no signs of maternal toxicity and no signs of embryo/fetal toxicity up to and including a dose of 250 mg/kg bw per day. There were no indications of teratogenic effects that could be causally related to the test substance administration.

Bentazone technical at a dose of 250 mg/kg bw per day induced some signs of maternal (reduced feed consumption, impaired body weight gains) and developmental (reduced fetal body weights and delayed maturation of the fetal skeletons) toxicity, but no teratogenic effects were seen (Becker et al., 1987a).

For this prenatal toxicity study with 8-hydroxybentazone in rats, the NOAEL for maternal and fetal toxicity was 250 mg/kg bw per day, whereas the NOAEL was 100 mg/kg bw day in the preceding toxicity study (Becker et al., 1987a) with bentazone technical, the parent compound.

The study was GLP compliant, and a QA statement was attached (Hellwig & Hildebrand, 1993).

(c) Dermal penetration

Rats in vivo

The absorption and elimination of bentazone (purity 97%) have been studied in Sprague-Dawley rats after dermal application of [^{14}C]bentazone sodium salt in an aqueous solution at a concentration of 0.002, 0.02, 0.2 or 2 mg/cm^2 as the free acid, which correspond to dermally applied doses of 0.12, 1.2, 12 and 120 mg/kg bw, respectively.

At each dose level, groups of four animals were sacrificed at 0.5, 1, 2, 4 and 10 hours after dosing. The remaining four rats per group were transferred to metabolism cages after coverings had been removed, and their backs were washed with methanol at 10 hours after dose application. From these animals, samples of urine and faeces were collected during intervals of 10–24, 24–48 and 48–72 hours. At 72 hours after dosing, rats were killed, and samples of blood and tissues were taken for analysis. In addition, concentrations of radioactivity in liver and plasma and excretion of radioactivity in urine and faeces after oral administration of bentazone sodium salt at a dose level equal to 4 mg/kg bw as the free acid to male rats were determined. The orally dosed animals were allocated to groups of four per dose level and sacrificed at the same times after dosing as indicated for the dermal application groups. Rats scheduled for termination after 72 hours were housed in metabolism cages. Urine and faeces were collected separately during intervals of 0–6, 6–12, 12–24, 24–48 and 48–72 hours. The animals received standardized diet and water ad libitum.

Only small proportions of the dose (1.23%, 1.90%, 1.46% and 0.79%, respectively) were absorbed at dermally applied concentrations of 0.002, 0.02, 0.2 and 2 mg/cm^2. This dermally absorbed part was rapidly eliminated, with urinary excretion being the main route. Less than 0.1% of the dose was excreted via the faeces within 10 hours. After a 4 mg/kg bw oral dose of [^{14}C]bentazone sodium salt, a mean of 90% of the total dose was excreted in the urine during 72 hours. A major part (85%) was excreted during the first 12 hours. These results clearly indicated that the oral dose was extensively and rapidly absorbed and eliminated. The time course of radioactive plasma levels at the different times confirms the limited extent of percutaneous absorption.

The proportion of the dose remaining in skin and fur was much greater at lower dose levels. Means of 61%, 28%, 13% and 2%, respectively, remained, after washing with cotton wool swabs moistened with water, on the treated skin of animals sacrificed at 72 hours after application of the 0.002, 0.02, 0.2 and 2 mg/cm^2 doses, respectively. This finding might suggest that a certain amount of test compound could not be easily removed from the skin or fur.

Mean concentrations of radioactivity in tissues after dermal application of 0.12, 1.2 and 12 mg/kg bw were generally low and mostly below the level of quantification. The concentrations in the eye, brain and testes after dermal application were low and frequently below the level of quantification.

Mean concentrations of radioactivity in plasma after the 4 mg/kg bw oral dose were 7.07, 6.29, 3.46, 1.84, 0.166 and 0.0041 µg/ml. The concentrations of radioactivity in liver were 2.46, 1.94, 1.25, 0.789, 0.117 and < 0.0091 µg/g, respectively.

In view of the above, it can be concluded that only about 1–2% of the amount applied dermally was absorbed. In contrast, 90% of the test substance administered orally was excreted with the urine within 72 hours, confirming the high degree of absorption following oral administration. Seventy-two hours after dermal application of even the highest dose, only traces of radioactivity were present in the animal body. Elimination of the dermally absorbed amount was rapid and effective, with the urine being the main route. It has been stated that although GLP was not compulsory when the study was performed, the study was run according to the principles of GLP (Hawkins et al., 1986b).

Human skin in vitro

The dermal penetration of [^{14}C]bentazone sodium salt (batch no. 210-2201, radiochemical purity 97.3%) through human skin was assessed by a single topical application of about 4933, 49.3 or

8.22 μg/cm^2 of active ingredient formulated in BAS 351 32 H to split thickness skin membranes mounted on Franz-type diffusion cells. The doses represent the formulation concentrate or two representative spray dilutions (1:100 and 1:600) for field use, respectively. The study was performed using five diffusion cells per dose.

Diffusion cells were operated in the static mode with tap water as the receptor fluid. The openings of the donor compartments were covered with Fixomull® stretch adhesive fleece (semi-occlusive conditions) after application. After a 6-hour exposure period, the surface of the skin membranes was washed. The adhesive fleece cover of the donor compartment was reconstituted, and the study continued up to 24 hours. During the study period, amounts of the receptor fluid were collected from each cell at several time points in order to determine kinetic parameters (lag phase, absorption rate and permeability constant). At the end of the sampling period, the test substance was recovered from all compartments of each diffusion cell. The recovery results are summarized as non-absorbed dose (skin washing and tape stripping), amount associated with the skin membrane and absorbed dose (receptor fluid, receptor chamber washing and receptor samples including washout). The mean recovery rates and absorption kinetic parameters are presented in Table 22.

Table 22. Recovery results in a human skin dermal penetration study

Parameter		Group 1	Group 2	Group 3
Target applied dose of test preparation	(mg or μl/cm^2)	10	10	10
Target applied dose of test substance	(μg/cm^2)	4933	49.3	8.22
Mean nominal applied dose of test substance	(μg/cm^2)	5020	51.2	8.47
Recovery				
Mean total recovery rate	(% of applied dose)	101.7	98.5	96.2
Mean non-absorbed dose	(% of applied dose)	101.6	97.7	95.3
Mean amount associated with skin	(% of applied dose)	0.04	0.50	0.57
Mean absorbed dose	(% of applied dose)	0.00	0.30	0.30
Absorption kinetics				
K_p	($\times 10^{-5}$ cm/h)	—[a]	0.236[b]	1.01[b]
Absorption rate	(μg/(cm^2·h))	—[a]	0.012[b]	0.008[b]
Lag time	(h)	—[a]	1.8[b]	2.5[b]

From Gamer & Landsiedel (2009)
K_p, permeability coefficient
[a] The absorbed dose was too low for meaningful calculation of kinetic parameters.
[b] Kinetic parameters are doubtful due to low absorbed dose.

The mean total recovery rates fulfil the quality criteria put forward in the test guidelines.

No meaningful absorbed doses were measured in diffusion cells treated with the high dose. The mean absorbed dose was very low for the middle and low doses also (0.30% each). Also, the amount of test substance associated with the skin membranes was very low (0.04%, 0.50% and 0.57% for the high, middle and low doses, respectively).

Summing up the absorbed dose with that associated with the skin membrane, 0.04%, 0.80% and 0.87% of the applied dose were recovered from diffusion cells for the high, middle and low doses, respectively. For the high dose, no cumulative absorbed dose curves could be generated. Consequently, no absorption rate, permeability coefficient or lag time could be calculated for this dose.

The receptor samples start to show quantifiable amounts of radioactivity (test substance) 2–4 hours after application in the mid-dose group and 2–6 hours after application in the low-dose group.

Therefore, the mean cumulative absorbed dose curves of these test groups show the steepest slopes between 1 and 2 hours after application, and the curves show an unsteady and decreasing slope thereafter. Although for the middle and low doses cumulative absorbed dose curves could be generated, the kinetic parameters calculated from these curves are doubtful due to the very low absorbed doses obtained.

Within the variability of the method, the absorption rates for the middle and low doses are comparable and do not reflect the dilution factor between these doses.

Consequently, the permeability coefficient of the low-dose group is higher than that for the mid-dose group. The absorption lag times calculated in the mid- and low-dose groups were 1.8 and 2.5 hours, respectively, and show the presence of a functional barrier in the skin samples used. According to the categorization schemes suggested by Marzulli, Brown & Maibach (1969) and Barber, Hill & Schum (1995), the permeability coefficients derived from the steepest parts of the penetration–time curves show a very slow to slow diffusion of [^{14}C]bentazone sodium salt from the spray dilutions through human skin membranes. For the formulation concentrate (high dose), virtually no diffusion of [^{14}C]BAS 351 H (bentazone sodium salt) through human skin membranes was present under the test conditions used.

In view of the above, it can be concluded that in vitro dermal penetration of bentazone formulated as an aqueous soluble (liquid) concentrate formulation of bentazone sodium through human skin is appropriately calculated as per cent absorbed dose. Considering the amount of radiolabelled substance associated with the skin (remaining skin and tape strips 3–6) after washing as absorbable and combining this with the absorbed amount detected in the receptor, the extent of dermal penetration through human epidermis is about 0.06% for the concentrate, 1.31% for the 1:100 spray strength dilution and 1.23% for the 1:600 dilution.

The study was GLP compliant, and a QA statement was attached (Gamer & Landsiedel, 2009).

3. Observations in humans

3.1 Medical surveillance of manufacturing plant personnel

All persons handling crop protection products are surveyed by regular medical examinations. There are no specific parameters available for effect monitoring of bentazone. Thus, the medical monitoring programme is designed as a general health checkup, with special interest in the primary target organs presumed to be relevant by analogy from animal experiments. The surveillance programme includes a general physical examination, including neurological status, red and white blood cell counts and liver enzymes. Adverse health effects suspected to be related to bentazone exposure have not been observed. Some cases of irritation of the eyes and the skin have been registered in the BASF internal clinical incident log in persons exposed to bentazone. No other adverse health effects due to bentazone have been documented in the BASF internal medical files.

At BASF sites in Germany and the USA, studies have been performed among employees who had been assigned to bentazone production facilities in the 1970s and 1980s. However, these studies do not address potential effects of the final product, but those of a specific multi-step batch process that included a large number of process starting materials and chemical intermediates and that is now obsolete (Nasterlack et al., 2007).

3.2 Direct observation

A literature search (24 October 2011) retrieved several case reports of suicide attempts with bentazone, some of which resulted in deaths:

- After ingestion of approximately 36 g bentazone for a suicide attempt, a 41-year-old man developed rhabdomyolysis with acute renal failure, vomiting, palpitation, fever and somnolence. Fifteen hours after intake, urine output decreased, and blood, urine nitrogen,

creatinine and creatinine kinase levels were increased. On the 5th day of hospitalization, laboratory findings had returned to normal levels (Emre et al., 2011).

- A 23-year-old healthy male farmer attempted to commit suicide by consumption of approximately 80 ml of bentazone (35.3 g, 569 mg/kg bw). He developed nausea, vomiting, cough, abdominal pain and nasogastric irritation and received a gastric lavage. The patient recovered and was discharged 5 days after admission. Another 31-year-old man, suffering from alcohol abuse and schizophrenia, ingested approximately 200 ml bentazone (88.2 g; 1.764 mg/kg bw). Icteric sclera and multiple reddish ulcers in the oral cavity, tongue base and posterior wall of the oropharynx were found. Over the next few days, the patient developed acute renal failure, fluid overloading and high anion gap metabolic acidosis; he died 5 days after admission (Wu et al., 2008).

- A 59-year-old woman who intentionally ingested 100–200 ml Basagran (about 50–100 g bentazone) was taken to the hospital with cardiac arrest 2 days after she had consumed the herbicide. During this period, she suffered vomiting, urination and diarrhoea, and she was drowsy with a muddled speech. Biological samples obtained at the autopsy were analysed, and the presence of bentazone, alcohol and an active metabolite of citalopram was detected. Blood concentrations of bentazone, alcohol and desmethyl-citalopram were 625 mg/kg, 0.62 g/l and 0.03 mg/kg, respectively (Müller et al., 2003).

- A case of fatal suicidal bentazone poisoning was presented along with a description of the different analytical methods involved. A 56-year-old farmer was examined by the family doctor 1 hour after voluntarily ingesting 500 ml of FIGHTER (about 250 g bentazone). He presented a Glasgow score of 15, polypnoea, diarrhoea and vomiting. During transport by ambulance to the hospital, he tossed, sweated and suddenly presented breathing difficulty followed by heart failure. The patient died within 2 hours post-ingestion. Blood and urine samples were taken just before death. Bentazone plasma and urine levels were 1500 and 1000 mg/l, respectively (Turcant et al., 2003).

- A 27-year-old robust man, without any medical or surgical history, attempted to commit suicide by consuming 300 ml Basagran (about 130 g bentazone). This poisoning resulted in vomiting, fever, sweating, pipe-like muscle rigidity, sinus tachycardia, drowsiness, leukocytosis, rhabdomyolysis and hepatorenal damage. Empirical treatment with bromocriptine was temporally associated with resolution of the above signs and symptoms. His clinical presentations and the effect of bromocriptine may be indicative that Basagran poisoning mimicks neuroleptic malignant syndrome (Lin et al., 1999).

There have been several unpublished reports of deaths after ingestion of bentazone. The lowest reported dose associated with a death was 20 g; however, this information is related to a case that occurred in China and could not be verified.

Comments

Biochemical aspects

Toxicokinetic studies performed on mice, rats and rabbits indicate that bentazone is rapidly and almost completely absorbed via the oral route (> 99%), and maximum blood concentrations of radioactivity are achieved in approximately 15 minutes at low doses (4 mg/kg bw) and by 1 hour at high doses (200 mg/kg bw). Administration of bentazone either as the sodium salt or as the free acid did not result in any significant differences in absorption. There was no evidence of penetration into the central nervous system or spinal cord, and elimination from other tissues was rapid, with no indication of bioaccumulation.

Elimination was almost exclusively via the urine (approximately 91% within 24 hours); 5 days after dosing, less than 2% was found in faeces and less than 0.02% in expired air. Biliary excretion of radioactivity was minimal. No significant differences were found in absorption and elimination among the different species investigated (rat, rabbit, mouse).

Bentazone is minimally metabolized in vivo, with the parent compound being the predominant excretion product. Only small amounts of 6-hydroxybentazone (up to approximately 6% of the dose) and minimal amounts of 8-hydroxybentazone (less than approximately 0.2% of the dose) were detected in urine.

Toxicological data

Bentazone has moderate acute toxicity when administered orally to rats, guinea-pigs and rabbits and low toxicity when administered dermally or by inhalation to rats. In rats, the LD_{50} was greater than or equal to 850 mg/kg bw. The dermal LD_{50} in rats was greater than 5000 mg/kg bw. The inhalation LC_{50} was greater than 5.1 mg/l of air (4-hour exposure; nose only). Bentazone was moderately irritating to the eye but not irritating to the skin in rabbits. It was a dermal sensitizer in the Magnusson and Kligman maximization test and the Buehler test in guinea-pigs.

Repeated-dose toxicity studies (subchronic and chronic) in mice, rats and dogs indicate that effects on haematology and blood coagulation (e.g. prolongation of prothrombin time and partial thromboplastin time) were consistently observed.

Three short-term oral rat studies demonstrated an overall NOAEL of 400 ppm (equal to 25.3 mg/kg bw per day), with a LOAEL of 800 ppm (equal to 40 mg/kg bw per day) for decreased body weight gain, decreased feed consumption, increased serum total cholesterol levels, increased urine output and prolonged prothrombin time and partial thromboplastin time.

In 90-day and 1-year dog studies, clinical signs, anaemia and effects on blood coagulation were noted. In the 90-day study, the NOAEL was 300 ppm (equal to 12.0 mg/kg bw per day), on the basis of sedation and ulceration and alopecia on the leg of one dog at 1000 ppm (equal to 39.6 mg/kg bw per day). The NOAEL for the 1-year study was 400 ppm (equal to 13.1 mg/kg bw per day), on the basis of anaemia, altered blood coagulation parameters, clinical signs and weight loss at the highest dietary concentration of 1600 ppm (equal to 52.3 mg/kg bw per day).

In a 2-year dietary toxicity and carcinogenicity study in mice, the NOAEL was 100 ppm (equal to 12 mg/kg bw per day), based on prolongation of prothrombin time and an increased incidence of calcification of the testicular tunica albuginea and deferent canals in the males at 400 ppm (equal to 47 mg/kg bw per day). No carcinogenic effects were observed in this study.

In a 2-year combined toxicity and carcinogenicity study in rats, the NOAEL was 200 ppm (equal to 9 mg/kg bw per day), based on clinical chemistry changes indicative of effects on liver and kidney and effects on blood coagulation parameters at 800 ppm (equal to 35 mg/kg bw per day). No carcinogenic effects were observed in this study.

The Meeting concluded that bentazone was not carcinogenic in rats or mice.

Bentazone was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. It showed no evidence of genotoxicity.

The Meeting concluded that bentazone is unlikely to be genotoxic.

In view of the lack of genotoxicity and the absence of carcinogenicity in rats and mice, the Meeting concluded that bentazone is unlikely to pose a carcinogenic risk to humans.

In a two-generation dietary reproduction study in rats, the NOAEL for parental and offspring toxicity was 200 ppm (equal to 14 mg/kg bw per day), on the basis of reduced parental feed consumption and body weight gain and reduced pup body weight resulting from parental toxicity at 800 ppm (equal to 59 mg/kg bw per day). There were no effects on reproduction at 3200 ppm (240 mg/kg bw per day), the highest dose tested.

In two studies of developmental toxicity in rats treated by gavage, the overall NOAEL for maternal toxicity was 250 mg/kg bw per day, the highest dose tested. The overall developmental NOAEL was 200 mg/kg bw per day, on the basis of increased post-implantation loss, reduced weight of fetuses surviving to day 21 and skeletal anomalies at the next higher dose of 250 mg/kg bw per day.

In a third study of developmental toxicity, in which rats were given diets containing bentazone from day 0 to day 21, the NOAEL for maternal toxicity was 2000 ppm (equal to 162 mg/kg bw per day), on the basis of increased water consumption at 4000 ppm (equal to 324 mg/kg bw per day). The developmental NOAEL was 4000 ppm (equal to 324 mg/kg bw per day), on the basis of decreased fetal weight gain and reduced ossification of cervical vertebrae at 8000 ppm (equal to 631 mg/kg bw per day).

In two gavage studies of developmental toxicity in rabbits, the overall NOAEL for maternal and developmental toxicity was 150 mg/kg bw per day, on the basis of a reduction in maternal feed consumption and increased post-implantation losses at 375 mg/kg bw per day.

The Meeting concluded that bentazone was not teratogenic in rats or rabbits.

In a subchronic neurotoxicity study, there was no indication of neurotoxicity at doses up to 3500 ppm (equal to 258 mg/kg bw per day), the highest dose tested.

6-Hydroxybentazone and 8-hydroxybentazone are major plant metabolites of bentazone. Both were less acutely toxic than the parent compound. Neither of the metabolites induced mutations in bacterial tests, and 8-hydroxybentazone was also not genotoxic in an in vitro mammalian forward mutation test and an in vivo mouse micronucleus test. In a subchronic dietary toxicity study and a developmental toxicity study in rats with 8-hydroxybentazone, the NOAEL was approximately 250 mg/kg bw per day, the highest dose tested.

No adverse health effects or poisoning in manufacturing plant personnel or in operators and workers exposed to bentazone have been reported.

Several case reports of suicide attempts due to ingestion of bentazone formulations have been reported in the literature, including four cases resulting in death. The range of doses ingested that resulted in death was 35–250 g of bentazone. The poisoning symptoms and signs included nausea, vomiting, abdominal pain, rhabdomyolysis, hepatorenal damage and cardiac failure.

The Meeting concluded that the existing database on bentazone was adequate to characterize the potential hazards to fetuses, infants and children.

Toxicological evaluation

The Meeting established an ADI of 0–0.09 mg/kg bw derived from a NOAEL of 9 mg/kg bw per day from the 2-year study of toxicity and carcinogenicity in rats, on the basis of prolonged blood coagulation and clinical chemistry changes indicative of effects on liver and kidney at 35 mg/kg bw per day. A safety factor of 100 was applied. This ADI was supported by the NOAEL of 13.1 mg/kg bw per day observed in the 1-year study in dogs for anaemia, altered blood coagulation parameters, clinical signs and weight loss seen at the highest dose of 52.3 mg/kg bw per day; by the NOAEL of 14 mg/kg bw per day in the two-generation study in rats, on the basis of reduced parental feed consumption and body weight gain and reduced pup body weight resulting from parental toxicity at 59 mg/kg bw per day; and by the NOAEL of 12 mg/kg bw per day in a 2-year toxicity and carcinogenicity study in mice, based on prolongation of prothrombin time and an increased incidence of testicular calcification at 47 mg/kg bw per day.

The Meeting reaffirmed its previous conclusion that no ARfD is necessary. It considered that the post-implantation loss seen in the rat developmental study was not caused by a single dose and that no other effects were observed in repeated-dose studies that could be due to a single dose.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year study of toxicity and carcinogenicity[a]	Toxicity	100 ppm, equal to 12 mg/kg bw per day	400 ppm, equal to 47 mg/kg bw per day
		Carcinogenicity	2000 ppm, equal to 242 mg/kg bw per day[b]	—
Rat	Short-term studies of toxicity[c]	Toxicity	400 ppm, equal to 25.3 mg/kg bw per day	800 ppm, equal to 40 mg/kg bw per day
	Two-year studies of toxicity and carcinogenicity[a,c]	Toxicity	200 ppm, equal to 9 mg/kg bw per day	800 ppm, equal to 35 mg/kg bw per day
		Carcinogenicity	4000 ppm, equal to 274 mg/kg bw per day[b]	—
	Two-generation study of reproductive toxicity[a]	Reproductive toxicity	3200 ppm, equal to 240 mg/kg bw per day[b]	—
		Parental toxicity	200 ppm, equal to 14 mg/kg bw per day	800 ppm, equal to 59 mg/kg bw per day
		Offspring toxicity	200 ppm, equal to 14 mg/kg bw per day	800 ppm, equal to 59 mg/kg bw per day
	Developmental toxicity studies[c,d]	Maternal toxicity	250 mg/kg bw per day[b]	—
		Embryo and fetal toxicity	200 mg/kg bw per day	250 mg/kg bw per day
Rabbit	Developmental toxicity study[d]	Maternal toxicity	150 mg/kg bw per day	375 mg/kg bw per day
		Embryo and fetal toxicity	150 mg/kg bw per day	375 mg/kg bw per day
Dog	Ninety-day and 1-year studies of toxicity[a,c]	Toxicity	400 ppm, equal to 13.1 mg/kg bw per day	1000 ppm, equal to 39.6 mg/kg bw per day

[a] Dietary administration.
[b] Highest dose tested.
[c] Two or more studies combined.
[d] Gavage administration.

Estimate of acceptable daily intake for humans

0–0.09 mg/kg bw

Estimate of acute reference dose

Unnecessary

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposure

Critical end-points for setting guidance values for exposure to bentazone

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapidly and almost completely absorbed (> 90%)
Dermal absorption	Poorly absorbed (1–2%)
Distribution	Widely distributed
Potential for accumulation	None
Rate and extent of excretion	Rapid, more than 90% within 24 h, mainly via urine
Metabolism in animals	Minimal
Toxicologically significant compounds in animals, plants and the environment	Parent compound
Acute toxicity	
Rat, LD_{50}, oral	≥ 850 mg/kg bw
Rat, LD_{50}, dermal	> 5000 mg/kg bw
Rat, LC_{50}, inhalation	> 5.1 mg/l of air
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Irritating
Dermal sensitization	Sensitizer (Magnusson & Kligman test)
Short-term studies of toxicity	
Target/critical effect	Blood coagulation
Lowest relevant oral NOAEL	12 mg/kg bw per day (dog)
Lowest relevant dermal NOAEL	1000 mg/kg bw per day (highest dose tested) (rabbit)
Lowest relevant inhalation NOAEC	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Blood coagulation, liver and kidney effects
Lowest relevant NOAEL	9 mg/kg bw per day (rat)
Carcinogenicity	Not carcinogenic in rats or mice
Genotoxicity	
	Not genotoxic
Reproductive toxicity	
Reproduction target/critical effect	No reproductive effects
Lowest relevant parental NOAEL	14 mg/kg bw per day (rat)
Lowest relevant offspring NOAEL	14 mg/kg bw per day (rat)
Lowest relevant reproductive NOAEL	240 mg/kg bw per day (highest dose tested) (rat)
Developmental toxicity	
Developmental target/critical effect	Post-implantation loss, reduced fetal weight and skeletal anomalies
Lowest relevant maternal NOAEL	150 mg/kg bw per day (rabbit)
Lowest relevant embryo/fetal NOAEL	150 mg/kg bw per day (rabbit)
Neurotoxicity	
Subchronic neurotoxicity	No effect up to 258 mg/kg bw per day (highest dose tested)

Medical data	
	No significant health effects in manufacturing personnel. Six cases of intentional poisoning have been reported with various critical symptoms.

Summary

	Value	Study	Safety factor
ADI	0–0.09 mg/kg bw	2-year chronic toxicity and carcinogenicity study (rat)	100
ARfD	Unnecessary	—	—

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CHLORFENAPYR

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Explanation

Chlorfenapyr is the International Organization for Standardization (ISO)–approved name for 4-bromo-2-(4-chlorophenyl)-1-ethoxymethyl-5-trifluoromethyl-1H-pyrrole-3-carbonitrile (International Union of Pure and Applied Chemistry) (Chemical Abstracts Service No. 122453-73-0). Chlorfenapyr is a contact and stomach insecticide that acts, following metabolic activation, as an uncoupler of oxidative phosphorylation in mitochondria. It has limited systemic activity.

Chlorfenapyr has not been evaluated previously by the Joint FAO/WHO Meeting on Pesticide Residues and was reviewed at the present Meeting at the request of the Codex Committee on Pesticide Residues.

All critical studies with chlorfenapyr were certified to be compliant with good laboratory practice (GLP), unless otherwise specified.

Evaluation for acceptable daily intake

1. Biochemical aspects

The absorption, distribution, metabolism and excretion of chlorfenapyr were investigated in rats. Two of the studies (Okada, 1994a,b) were not certified as being compliant with GLP.

The structural formulae of [2-pyrrole-^{14}C]chlorfenapyr and [phenyl(U)-^{14}C]chlorfenapyr are given in Figure 1.

Figure 1. Structural formulae of radioactively labelled chlorfenapyr used in toxicokinetic studies

* denotes position of ^{14}C or ^{13}C at the 2-position of the pyrrole ring [2-pyrrole-^{14}C]

▲ uniformly labelled ^{14}C phenyl ring [phenyl(U)-^{14}C]

1.1 Absorption, distribution and excretion

Groups of five male and five female Sprague-Dawley rats were treated with the radiolabelled compounds as follows:

- SOLD: single oral administration of low-dose labelled compound;
- SOHD: single oral administration of high-dose labelled compound;
- MOLD: daily oral administration of low-dose non-radioactive compound for 14 days, followed by single oral administration of low-dose labelled compound;
- Control: single oral administration of dosing vehicle.

The control group comprised three animals of each sex. The radiolabelled test substance was suspended in aqueous (0.5% weight per weight [w/w]) sodium salt of carboxymethylcellulose and administered orally by gavage.

The actual oral dose rate for each treatment group is shown in Table 1.

Table 1. Doses used in toxicokinetics study

Treatment	Dose (mg/kg bw)	
	[2-pyrrole-^{14}C]	[phenyl(U)-^{14}C]
SOLD	19.3	20.0
SOHD	208.3	205.1
MOLD	19.2	21.7
Control	—	—

From Mallipudi (1994)

bw, body weight; MOLD, multiple oral low dose; SOHD, single oral high dose; SOLD, single oral low dose

All urine, faeces and cage rinses were collected at the following time intervals: 0–4, 4–8, 8–12, 12–24, 24–36, 36–48, 48–72, 72–96, 96–120, 120–144 and 144–168 hours post-dosing. Selected tissue samples were collected at termination. Specific radioactivity was determined by high-performance liquid chromatography (HPLC) of the analyte and standard chlorfenapyr. Total radioactive residue (TRR) in the blood, tissues/organs and faeces or post-extraction residuum was determined by combustion followed by liquid scintillation counting. Radioactivity in the urine, as well as in liquid chromatographic column eluates, was measured by liquid scintillation counting.

The absorbed chlorfenapyr-related residue was distributed throughout the body and detected at concentrations ranging from 0.02 to 24.3 µg equivalents (Eq) chlorfenapyr per gram tissue in all tissue and organ matrices of all treatment groups. The mean residual percentage of administered radioactivity in blood, carcasses and tissues at 7 days post-dosing ranged from less than 0.01% to 3.37% (Table 2). The concentration of radioactive residue was 0.58–7.75 µg Eq chlorfenapyr per gram tissue in blood, 1.0–24.3 µg Eq chlorfenapyr per gram tissue in fat, 0.09–1.05 µg Eq chlorfenapyr per gram tissue in muscle, 0.34–3.10 µg Eq chlorfenapyr per gram tissue in kidneys and 0.90–6.69 µg Eq chlorfenapyr per gram tissue in liver. Brain showed the lowest concentration of radioactivity (0.02–0.19 µg Eq chlorfenapyr per gram tissue) among all tissues evaluated. Fat showed the highest concentration of radioactivity (1.0–24.3 µg Eq chlorfenapyr per gram tissue). The animal carcasses showed 0.23–3.08 µg Eq of radioactive residues per gram tissue (0.58–3.37% of the administered dose). In general, the highest concentration level in each tissue/organ was obtained from the high-dose groups. Sex-related differences were noted in residue levels for all dose groups. Concentrations were higher by 2- to 3-fold in female rats than in male rats in blood and most tissues, but were the same in liver.

A pilot metabolism study showed only trace amounts of radiocarbon (< 0.01% of the dose) in volatile organic compounds and no radioactivity in the expired carbon dioxide over a 7-day period. During the first 24 hours following administration of radiolabelled chlorfenapyr, approximately 70% of the dose was excreted (66% in faeces and 4% in urine). Within 48 hours after dosing, approximately 88% of the dose had been excreted (82% in faeces and 6% in urine). The administered oral dose was eliminated over a 7-day period mainly via faeces (80–106%) and to a much lesser extent via urine (5.3–11.2%), regardless of the treatment regimen or position of ^{14}C label.

The excretion of chlorfenapyr-related radioactivity in the rat is summarized in Table 3 (urine) and Table 4 (faeces).

In summary, there was no evidence of elimination of chlorfenapyr-related radioactivity via respiration. The principal route of elimination of orally administered chlorfenapyr was via faeces. There are no substantial ^{14}C label–related differences in the absorption, distribution or elimination of radioactivity in the rat. In general, the higher concentrations of radiocarbon in the tissues and organs were obtained with the high-dose treatment. Blood and tissue radiocarbon concentrations appeared higher in female rats than in male rats (Mallipudi, 1994).

In another study on the disposition of chlorfenapyr, groups of four male and four female Sprague-Dawley rats were treated as follows:

- low dose: 1.85 MBq/2 mg at a dosing volume of 4 ml/kg body weight (bw), single oral administration;
- high dose: 1.85 MBq/20 mg at a dosing volume of 4 ml/kg bw, single oral administration.

The radiolabelled test substance was administered orally by gavage as an aqueous solution in 1% sodium carboxymethylcellulose plus 1% Tween 80. Blood was sampled at 15 and 30 minutes and 1, 2, 4 , 8, 12, 24, 48, 72, 120 and 168 hours post-dosing. Urine was collected 6 and 12 hours post-dosing. Urine and faeces were collected at 24, 48, 72, 120 and 168 hours post-dosing. Organs and tissues from four animals of each sex were collected at 1, 8, 24 and 168 hours post-dosing. In bile duct–cannulated animals, bile was collected at 3, 6, 12 and 24 hours; urine was collected at 6, 12 and 24 hours; and faeces was collected at 24 hours post-dosing.

Table 2. Mean radioactivity detected in blood carcass and tissue samples 7 days following dosing with ^{14}C-labelled chlorfenapyr

Matrix	% of radioactive dose in matrix					
	SOLD		MOLD		SOHD	
	M	F	M	F	M	F
[2-pyrrole-^{14}C]						
Blood	0.08	0.21	0.10	0.32	0.04	0.10
Bone (femur)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Brain	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Carcass	1.58	3.06	1.50	3.02	0.58	1.51
Fat (body)	0.14	0.26	0.07	0.14	0.03	0.17
Heart	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Kidneys	0.02	0.03	0.02	0.03	< 0.01	< 0.01
Liver	0.48	0.40	0.36	0.38	0.15	0.17
Lungs	< 0.01	0.03	0.01	0.03	< 0.01	< 0.01
Muscle (thigh)	< 0.01	0.02	< 0.01	0.03	< 0.01	< 0.01
Ovaries	NA	< 0.01	NA	< 0.01	NA	< 0.01
Skin (shaved)	0.03	0.05	0.03	0.07	0.01	0.04
Spleen	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Testes	0.02	NA	0.02	NA	< 0.01	NA
[phenyl(U)-^{14}C]						
Blood	0.07	0.22	0.06	0.22	0.04	0.08
Bone (femur)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Brain	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Carcass	1.41	3.37	1.12	2.63	0.76	1.38
Fat (body)	0.16	0.35	0.07	0.20	0.03	0.18
Heart	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Kidneys	0.02	0.03	0.01	0.03	< 0.01	< 0.01
Liver	0.37	0.38	0.28	0.31	0.17	0.15
Lungs	< 0.01	0.02	< 0.01	0.02	< 0.01	< 0.01
Muscle (thigh)	< 0.01	0.03	< 0.01	0.02	< 0.01	< 0.01
Ovaries	NA	< 0.01	NA	< 0.01	NA	< 0.01
Skin (shaved)	0.02	0.06	0.02	0.05	0.01	0.03
Spleen	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Testes	0.01	NA	< 0.01	NA	< 0.01	NA

From Mallipudi (1994)
F, female; M, male; MOLD, multiple oral low dose; NA, not applicable; SOHD, single oral high dose; SOLD, single oral low dose

After pyrrole-^{14}C-labelled chlorfenapyr was administered orally, ^{14}C began to be detected in the blood of the systemic circulation as early as 15 minutes, the first measurement interval; levels increased slowly, with a peak concentration in plasma (C_{max}) occurring at 8–12 hours. Based on the results, it was inferred that absorption of chlorfenapyr from the digestive tract was relatively slow. Concentrations of radiolabel declined slowly and monophasically, decreasing to about 7–13% of C_{max} by 168 hours, with ^{14}C-labelled chlorfenapyr gradually translocating from the systemic circulation to various organs and tissues. The plasma elimination half-life of radiolabel was approximately 56 hours.

Table 3. Cumulative radioactivity detected in urine following dosing with ^{14}C-labelled chlorfenapyr

Collection interval (h)	% of radioactive dose in urine[a]					
	SOLD		MOLD		SOHD	
	M	F	M	F	M	F
[2 -pyrrole-^{14}C]						
0–4	0.2	0.2	0.9	1.0	0.1	< 0.1
0–8	1.3	1.3	3.0	2.9	0.2	0.8
0–24	6.3	4.5	7.1	7.4	1.7	2.1
0–48	8.8	6.4	9.1	9.7	3.9	4.4
0–72	9.7	7.3	9.8	10.3	4.4	4.9
0–168	11.2	8.5	11.1	11.2	5.3	6.0
[phenyl(U)-^{14}C]						
0–4	0.8	0.9	1.2	1.3	0.7	0.7
0–8	2.3	2.1	2.9	3.1	1.0	1.3
0–24	6.4	4.7	6.6	6.9	3.2	4.2
0–48	8.4	6.4	7.8	8.6	6.1	5.7
0–72	9.2	7.0	8.4	9.1	6.7	6.1
0–168	10.4	8.1	9.4	10.2	7.6	6.8

From Mallipudi (1994)

F, female; M, male; MOLD, multiple oral low dose; SOHD, single oral high dose; SOLD, single oral low dose

[a] Includes cage rinses from 0- to 144-hour samples plus cage wash and cage wipe from 144- to 168-hour samples.

Table 4. Cumulative radioactivity detected in faeces following dosing with ^{14}C-labelled chlorfenapyr

Collection interval (h)	% of radioactive dose in faeces					
	SOLD		MOLD		SOHD	
	M	F	M	F	M	F
[2- pyrrole-^{14}C]						
0–4	NS	NS	NS	ND	< 0.1	NS
0–8	NS	NS	< 0.1	NS	< 0.1	1.7
0–24	66.7	57.1	77.1	63.2	65.5	57.7
0–48	84.5	74.7	90.9	89.6	83.0	78.6
0–72	89.0	82.4	94.9	96.8	85.4	84.0
0–168	93.7	92.0	99.4	106.0	87.8	88.5
[phenyl(U)-^{14}C]						
0–4	NS	NS	NS	ND	< 0.1	NS
0–8	NS	NS	NS	NS	< 0.1	4.4
0–24	62.3	57.4	79.2	68.0	60.1	72.6
0–48	76.5	70.4	85.7	80.3	74.9	91.4
0–72	81.0	76.2	88.1	86.0	78.1	94.7
0–168	86.1	83.9	91.4	92.7	80.1	97.5

From Mallipudi (1994)

F, female; M, male; MOLD, multiple oral low dose; ND, not detectable; NS, no sample; SOHD, single oral high dose; SOLD, single oral low dose

Maximum concentrations of chlorfenapyr in the liver, adrenals and fat were higher than those detected in plasma, with most tissues showing maximum levels between 1 and 8 hours. The per cent tissue distribution was elevated in tissues such as fat, muscle, skin and liver. Carbon-14 distributed among tissues did not show any tendency to remain there, and concentrations declined rapidly, with tissue concentrations decreasing to less than 10% of the maximum by 168 hours.

The radioactivity found in selected tissues of rats following administration of the low and high doses is summarized in Tables 5 and 6, respectively. Blood, plasma, fat, muscle and skin weights were assumed to be 7%, 4%, 5%, 40% and 22% of body weight, respectively.

The administered oral dose was almost completely excreted into the urine and faeces over a 168-hour period. Residues in tissues and carcass at 168 hours represented 2–5% of the oral dose. The main excretion route was faecal (75–85%), which was 5–10 times the excretion via the urinary route (8–16%). In bile duct–cannulated animals within the first 24 hours, between 2% and 19% of the ^{14}C was excreted into the faeces, between 4% and 6% into the urine and between 17% and 30% into the bile (Table 7). Cumulative excretion of ^{14}C into the bile was 3–7 times higher than that into the urine, suggesting that biliary excretion was the main route of ^{14}C excretion after absorption from the digestive tract. Faecal excretion thus included both the unabsorbed ^{14}C portion of the oral dose and biliary ^{14}C. It is likely that a portion of biliary ^{14}C entered the enterohepatic circulation.

Table 5. Summary of the radioactivity found in selected tissues of rats administered ^{14}C-labelled chlorfenapyr at 2 mg/kg bw

Tissue	Tissue radioactivity (% of dose)							
	Males (*n* = 4 at each time point)				Females (*n* = 4 at each time point)			
	1 h	8 h	24 h	168 h	1 h	8 h	24 h	168 h
Blood	2.03	4.18	2.79	0.49	2.34	3.68	3.10	0.54
Plasma	1.89	4.25	2.74	0.47	2.15	3.68	3.04	0.51
Thyroid	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Lung	0.12	0.13	0.07	0.01	0.16	0.14	0.10	0.02
Liver	5.66	3.19	2.07	0.58	7.13	3.29	2.35	0.54
Adrenal	0.02	0.01	< 0.01	< 0.01	0.04	0.01	0.01[a]	< 0.01
Kidney	0.40	0.39	0.25	0.05	0.47	0.40	0.29	0.04
Spleen	0.03	0.02	0.01	< 0.01	0.04	0.03	0.02	< 0.01
Pancreas	0.08	0.06	0.03	0.01[a]	0.15	0.12	0.06	0.02[b]
Fat	5.01	16.4	11.7	1.05	5.12	14.1	19.3	1.81
Brown fat	0.07	0.15	0.05	0.01[b]	0.11	0.26	0.11	0.01[a]
Muscle	4.29	2.90	1.62	0.28	6.67	3.71	2.40	0.30
Skin	3.53	7.44	3.48	0.56	4.89	9.42	6.30	0.80
Prostate gland/uterus	0.02	0.02	0.02	< 0.01	0.03	0.04	0.04	0.01
Stomach[c]	36.1	0.26	0.07	0.02	22.5	0.52	0.13	0.02
Small intestine[c]	40.5	8.77	2.22	0.14	37.8	7.63	2.36	0.20
Caecum[c]	0.10	27.7	6.98	0.16	2.57	40.7	7.30	0.27
Large intestine[c]	0.11	17.5	4.25	0.13	0.24	8.84	3.89	0.28

From Okada (1994a)

[a] Mean of three animals, when tissue content was < 0.01% in all other animals of the group.

[b] Mean of two animals, when tissue content was < 0.01% in all other animals of the group.

[c] Including contents.

Table 6. Summary of the radioactivity found in selected tissues of rats administered ^{14}C-labelled chlorfenapyr at 20 mg/kg bw

Tissue	Tissue radioactivity (% of dose)							
	Males (*n* = 4 at each time point)				Females (*n* = 4 at each time point)			
	1 h	8 h	24 h	168 h	1 h	8 h	24 h	168 h
Blood	0.95	2.12	2.06	0.33	0.89	3.37	3.02	0.33
Plasma	0.90	2.14	2.01	0.30	0.82	3.39	3.15	0.30
Thyroid	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Lung	0.08	0.07	0.05	0.01	0.08	0.13	0.10	0.01
Liver	3.98	2.00	1.65	0.39	4.02	3.61	1.92	0.37
Adrenal	0.02	0.01[a]	< 0.01	< 0.01	0.02	0.02	0.01[b]	< 0.01
Kidney	0.27	0.17	0.13	0.03	0.23	0.40	0.25	0.03
Spleen	0.02	0.02	0.01	< 0.01	0.02	0.02	0.02	< 0.01
Pancreas	0.07	0.06	0.03	< 0.01	0.09	0.16	0.07	< 0.01
Fat	3.97	15.6	8.54	0.21	2.62	14.2	15.4	0.67
Brown fat	0.05	0.20	0.03	< 0.01	0.06	0.33	0.14	< 0.01
Muscle	3.39	2.04	1.24	0.17	3.57	4.03	2.73	0.19
Skin	2.83	6.79	2.68	0.31	2.84	12.4	8.59	0.42
Stomach[c]	30.7	0.27	0.19[b]	0.02[a]	41.4	2.03	0.10	0.01[b]
Small intestine[c]	62.3	5.01	1.07	0.08	55.2	9.36	2.89	0.12
Caecum[c]	0.06	43.2	1.77	0.10	0.05	35.1	10.2	0.16
Large intestine[c]	0.06	13.6	2.66	0.08	0.07	14.1	3.53	0.17

From Okada (1994a)
[a] Mean of two animals, when tissue content was < 0.01% in all other animals of the group.
[b] Mean of three animals, when tissue content was < 0.01% in all other animals of the group.
[c] Including contents.

Table 7. Summary of the cumulative radioactivity found in bile, urine and faeces (bile duct–cannulated rats)

Sampling time (h)	Cumulative excreted radioactivity (% of dose)					
	2 mg/kg bw			20 mg/kg bw		
	Bile	Urine	Faeces	Bile	Urine	Faeces
Males						
0–3	4.1 ± 4.81	—	—	1.8 ± 1.54	—	—
0–6	9.5 ± 7.34	0.9 ± 0.62	—	4.8 ± 2.81	0.6 ± 0.32	—
0–12	18.3 ± 8.24	1.5 ± 0.59	—	10.7 ± 6.80	1.1 ± 0.56	—
0–24	30.1 ± 11.3	4.0 ± 1.25	9.7 ± 9.58	17.4 ± 9.14	5.5 ± 3.42	18.8 ± 15.5
Females						
0–3	4.7 ± 3.07	—	—	1.4 ± 0.60	—	—
0–6	11.5 ± 4.22	0.8 ± 0.59	—	4.7 ± 0.94	0.8 ± 0.47	—
0–12	19.6 ± 7.73	2.0 ± 1.23	—	9.8 ± 2.61	1.8 ± 0.77	—
0–24	24.1 ± 8.03	4.8 ± 2.29	2.3 ± 2.40	19.9 ± 7.35	4.4 ± 1.70	10.8 ± 6.31

From Okada (1994a)
—, not assayed

In summary, the absorption of orally administered chlorfenapyr was relatively slow, and translocation from the systemic circulation to tissues was gradual. More than 90% of the administered dose of chlorfenapyr was excreted into the faeces and urine by 168 hours. The principal route of elimination was via faeces, whereas the urinary route was minor. Faecal excretion included both the unabsorbed portion of the oral dose and the biliary fraction. Biliary excretion represented the main route of excretion after absorption from the digestive tract. It is also likely that a portion of the biliary excreta entered the enterohepatic circulation. No sex-related differences were observed in the described findings (Okada, 1994a).

In another study, groups of four male and four female Sprague-Dawley rats were treated as follows:

- low dose: 1.85 MBq/2 mg at a dosing volume of 4 ml/kg bw, single oral administration;
- high dose: 1.85 MBq/20 mg at a dosing volume of 4 ml/kg bw, single oral administration.

The radiolabelled test substance was administered orally by gavage as an aqueous solution in 1% sodium carboxymethylcellulose plus 1% Tween 80. Urine was sampled at 12, 24, 48 and 72 hours post-dosing. Faeces was collected at 24, 48 and 72 hours post-dosing. In bile duct–cannulated animals, bile was collected at 3, 6, 12 and 24 hours; urine was collected at 6, 12 and 24 hours; and faeces was collected at 24 hours post-dosing.

Almost all ^{14}C detected in faeces was unchanged chlorfenapyr. There was no unchanged chlorfenapyr in bile. This finding indicated that the unchanged chlorfenapyr in the faeces did not come from the bile, but consisted of chlorfenapyr that was not absorbed from the gastrointestinal tract and was directly excreted into the faeces. It follows, therefore, that the per cent absorption of chlorfenapyr from the gastrointestinal tract can be obtained by subtracting the percentage of faecal excretion of chlorfenapyr from the administered dose:

- male rats (2 mg/kg bw): 100% − 17.0% = 83.0%
- female rats (2 mg/kg bw): 100% − 23.1% = 76.9%
- male rats (20 mg/kg bw): 100% − 35.2% = 64.8%
- female rats (20 mg/kg bw): 100% − 33.0% = 67.0%

In summary, the per cent absorption of chlorfenapyr from the gastrointestinal tract was approximately 80% and 65% in the 2 mg/kg bw and 20 mg/kg bw groups, respectively, with an apparent decrease in absorption with increasing dose. Chlorfenapyr was absorbed unchanged; there was no evidence that degradation occurred in the digestive tract (Okada, 1994b).

1.2 Biotransformation

In the Mallipudi (1994) study described in section 1.1, the urinary residue consisted of M-4 along with sulfate and amino acid or peptide conjugates of M-4 and some minor unknown polar metabolites; the parent compound and its *N*-dealkylated product (M-8) were not detected in the urine (Table 8).

Faecal radiocarbons were composed mainly of unchanged chlorfenapyr (M-9), which accounted for 38–72% of the administered dose 48 hours after dosing. There were several identified minor metabolites (Table 9), each of which accounted for less than 5% of the dose over the same time period. The metabolites included the *N*-dealkylated product (M-8), M-4 (a 4-hydroxy-5-carboxypyrrole derivative of M-8), M-5 (a 4-oxo-5-hydroxypyrrole derivative of M-8), M-7A-RAT (a carboxymethylmethoxy derivative of M-9), M-6 (a desbromo derivative of M-7A-RAT) plus many minor polar unknowns.

Table 8. Summary of radioactive residue components in rat urine

Group/component	Residue in urine (% of dose)											
	[2-Pyrrole-^{14}C]						[Phenyl(U)-^{14}C]					
	0–12 h		12–24 h		24–48 h		0–12 h		12–24 h		24–48 h	
	M	F	M	F	M	F	M	F	M	F	M	F
SOLD												
Total	*3.3*	*2.4*	*3.0*	*2.1*	*2.5*	*1.9*	*4.1*	*3.1*	*2.3*	*1.6*	*2.0*	*1.7*
M-1	0.09	0.05	0.10	0.05	0.05	0.04	0.37	0.30	0.17	0.11	0.07	0.08
U-2	0.21	0.09	0.18	0.06	0.07	0.06	0.17	0.10	0.08	0.04	0.09	0.05
M-1A	0.35	0.15	0.10	0.04	0.24	0.17	0.12	0.11	0.10	0.03	0.08	0.05
M-2	0.72	0.26	0.68	0.29	0.40	0.22	1.36	0.65	0.62	0.27	0.32	0.32
M-3	0.39	0.25	0.47	0.20	0.53	0.23	0.31	0.29	0.20	0.14	0.51	0.21
M-4	0.45	0.27	0.29	0.21	0.24	0.21	0.72	0.53	0.26	0.20	0.18	0.17
M-5	0.54	0.86	0.77	0.90	0.76	0.68	0.65	0.79	0.64	0.62	0.54	0.56
M-6	0.09	0.07	0.12	0.08	0.08	0.07	0.05	0.06	0.05	0.02	0.06	0.02
Unknowns	0.45	0.29	0.25	0.24	0.15	0.18	0.25	0.20	0.13	0.12	0.11	0.13
MOLD												
Total	*5.0*	*4.7*	*2.1*	*2.7*	*2.0*	*2.3*	*5.0*	*4.8*	*1.6*	*2.1*	*1.2*	*1.7*
M-1	0.06	0.07	0.03	0.07	0.02	0.09	0.04	0.05	0.01	0.02	0.01	0.02
U-2	0.24	0.15	0.09	0.05	0.05	0.03	0.31	0.23	0.11	0.15	0.02	0.08
M-1A	0.36	0.39	0.15	0.25	0.19	0.23	0.92	0.60	0.18	0.24	0.04	0.18
M-2	0.77	0.37	0.30	0.28	0.27	0.24	0.75	0.57	0.37	0.24	0.13	0.26
M-3	0.66	0.50	0.34	0.31	0.38	0.26	0.20	0.24	0.08	0.15	0.27	0.10
M-4	1.06	0.82	0.37	0.33	0.44	0.29	0.93	0.85	0.16	0.20	0.08	0.14
M-5	1.03	1.59	0.58	0.96	0.43	0.80	1.20	1.56	0.51	0.79	0.53	0.65
M-6	0.17	0.16	0.07	0.11	0.06	0.13	0.17	0.19	0.06	0.08	0.04	0.08
Unknowns	0.59	0.51	0.15	0.28	0.14	0.21	0.44	0.46	0.10	0.21	0.08	0.18
SOHD												
Total	*0.5*	*1.4*	*1.2*	*0.7*	*1.6*	*1.5*	*1.5*	*3.2*	*1.7*	*1.0*	*2.9*	*1.5*
M-1	0.01	0.02	0.01	0.01	0.12	0.11	0.22	0.62	0.19	0.10	0.16	0.11
U-2	0.02	0.04	0.05	0.29	0.06	0.03	—	—	—	—	—	—
M-1A	0.02	0.02	0.19	0.08	0.14	0.10	0.07	0.09	0.09	0.05	0.15	0.09
M-2	0.13	0.32	0.14	0.07	0.40	0.23	0.48	0.93	0.50	0.18	0.86	0.24
M-3	0.05	0.11	0.10	0.05	0.19	0.08	0.09	0.15	0.17	0.06	0.29	0.11
M-4	0.07	0.26	0.18	0.14	0.09	0.18	0.32	0.72	0.18	0.12	0.25	0.13
M-5	0.09	0.32	0.28	0.19	0.42	0.55	0.15	0.39	0.32	0.33	0.62	0.55
M-6	0.02	0.05	0.06	0.04	0.02	0.05	0.02	0.05	0.04	0.02	0.04	0.03
Unknowns	0.06	0.22	0.17	0.09	0.13	0.17	0.13	0.19	0.19	0.10	0.47	0.21

From Mallipudi (1994)
—, not detected; F, female; M, male

Table 9. Summary of radioactive residue components in rat faeces

Group/ component	Residue in faeces (% of dose)											
	[2-Pyrrole-^{14}C]						[Phenyl(U)-^{14}C]					
	0–12 h		12–24 h		24–48 h		0–12 h		12–24 h		24–48 h	
	M	F	M	F	M	F	M	F	M	F	M	F
SOLD												
Total	*4.0*	*9.9*	*62.7*	*47.2*	*17.8*	*17.6*	*11.8*	*14.1*	*50.5*	*43.3*	*14.2*	*13.0*
U-1 (polar)	0.02	0.02	3.92	1.94	1.85	0.34	0.10	0.04	3.38	0.77	2.88	1.46
M-1	< 0.01	< 0.01	—	—	0.11	0.13	—	—	—	0.16	0.31	0.43
U-2	< 0.01	< 0.01	0.58	0.27.	0.32	0.71	0.08	—	0.64	—	0.43	0.96
M-1A	0.02	0.06	0.27	0.60	0.34	1.03	0.08	0.04	0.81	0.39	0.54	0.53
M-2	0.03	0.05	1.93	0.94	1.28	1.04	0.11	0.08	1.69	0.99	0.81	0.92
M-3	0.08	0.17	3.80	1.41	1.15	0.85	0.16	0.22	1.34	1.47	0.75	0.83
M-4	0.06	0.09	2.65	1.67	1.74	1.34	0.17	0.28	1.74	1.22	0.64	0.85
M-5	0.08	0.16	2.62	2.00	1.76	1.65	0.29	0.37	1.70	0.93	0.70	0.80
M-6	0.04	0.10	1.39	1.01	0.87	0.80	0.17	0.11	1.28	0.53	0.51	0.62
M-7	0.05	0.12	2.57	1.60	1.52	2.11	0.15	0.18	1.35	1.29	1.03	1.54
M-7A	0.02	0.05	0.80	0.57	0.39	0.45	0.11	0.07	0.66	0.65	0.24	0.31
M-8	0.06	0.15	0.87	0.71	0.23	0.25	0.15	0.24	0.58	0.51	0.14	0.13
M-9 (parent)	3.32	8.58	35.29	29.97	1.53	3.61	9.60	12.44	29.76	32.43	1.50	1.43
MOLD												
Total	*50.5*	*30.3*	*26.6*	*32.9*	*13.8*	*26.4*	*56.7*	*25.1*	*21.8*	*42.9*	*6.5*	*12.3*
U-1 (polar)	0.53	0.57	0.81	1.29	1.64	3.34	0.88	0.26	0.74	0.93	1.54	1.33
M-1	—	—	0.03	0.19	0.08	0.05	0.11	0.14	0.09	0.26	0.03	0.03
U-2	0.39	0.22	0.52	0.54	0.13	0.50	0.06	0.18	0.50	0.92	0.08	0.25
M-1A	0.26	0.11	0.88	0.85	0.50	0.83	0.45	0.19	0.76	0.51	0.17	0.43
M-2	1.00	0.24	1.43	1.52	1.29	1.16	0.63	0.33	0.51	1.55	0.37	0.63
M-3	1.87	0.35	1.40	1.05	0.76	1.46	1.99	1.02	1.56	2.35	0.50	0.63
M-4	1.67	1.03	2.33	2.50	1.57	2.92	1.68	0.89	1.41	2.80	0.48	1.18
M-5	1.84	0.76	2.23	2.30	1.09	2.15	1.14	0.50	1.28	2.15	0.47	1.44
M-6	1.22	0.42	0.99	1.81	0.55	1.30	0.97	0.43	0.81	1.32	0.37	1.01
M-7	1.59	0.84	1.99	2.31	1.38	2.04	0.73	0.39	0.84	1.93	0.40	1.03
M-7A	0.73	0.33	0.61	0.69	0.09	0.15	0.75	—	0.48	0.50	0.15	0.21
M-8	0.92	0.60	0.61	0.62	0.30	0.55	0.79	0.30	0.30	0.72	0.17	0.18
M-9 (parent)	33.68	22.70	8.67	13.08	1.15	2.71	43.57	19.13	9.01	22.25	0.36	1.10
SOHD												
Total	*26.3*	*28.2*	*39.2*	*40.5*	*17.5*	*20.9*	*24.5*	*51.5*	*35.6*	*21.1*	*14.8*	*18.8*
U-1 (polar)	0.12	0.03	0.22	0.15	0.66	0.51	0.07	0.02	0.92	0.31	1.41	0.87
M-1	—	< 0.01	—	0.03	0.23	0.06	< 0.01	0.01	0.04	0.02	0.08	0.05
U-2	< 0.01	< 0.01	0.21	0.12	0.12	0.44	0.01	0.06	0.07	0.12	0.09	0.14
M-1A	0.01	< 0.01	0.11	0.11	0.52	0.33	0.08	0.02	0.17	0.15	0.37	0.33
M-2	0.02	0.01	0.43	0.37	0.88	0.76	0.12	0.20	0.43	0.17	1.17	1.19
M-3	0.17	0.09	0.86	0.57	1.46	1.52	0.16	0.25	0.70	0.31	1.15	1.32
M-4	0.03	0.05	1.05	0.42	1.51	1.40	0.13	0.12	0.42	0.23	1.12	1.09

Group/ component	Residue in faeces (% of dose)											
	[2-Pyrrole-^{14}C]						[Phenyl(U)-^{14}C]					
	0–12 h		12–24 h		24–48 h		0–12 h		12–24 h		24–48 h	
	M	F	M	F	M	F	M	F	M	F	M	F
M-5	0.13	0.04	0.99	0.30	1.43	2.39	0.19	0.14	0.50	0.25	1.17	1.26
M-6	0.11	0.03	0.57	0.25	0.63	1.03	0.14	0.26	0.33	0.18	0.69	0.58
M-7	0.09	0.07	2.02	0.36	1.66	1.95	0.19	0.21	0.57	0.30	1.51	1.47
M-7A	0.08	0.06	0.35	0.12	0.35	0.53	0.19	0.26	0.54	0.10	0.34	0.34
M-8	0.35	0.39	0.63	0.52	0.19	0.26	0.36	0.71	0.45	0.31	0.19	0.20
M-9 (parent)	24.25	26.86	34.24	35.58	3.47	5.87	22.0	47.34	28.92	17.94	2.27	7.15

From Mallipudi (1994)

—, not detected; F, female; M, male

Analysis of tissue extracts showed that fat contained mainly unchanged chlorfenapyr (65.8–93.9% of the TRR; 4.37–22.82 µg Eq chlorfenapyr per gram tissue), whereas muscle, kidney and liver also contained more polar metabolites, which included M-8 (0.6–1.3% of the TRR), M-7, M-5, M-4, M-7A-RAT and M-6. Chlorfenapyr (M-9) and M-8 constituted 1.0–5.8% (0.03–0.18 µg Eq/g tissue) and 1.9–5.3% (0.03–0.15 µg Eq/g tissue) of the kidney TRR. Chlorfenapyr (M-9) and M-8 constituted 2.9–8.3% (0.15–0.51 µg Eq/g tissue) and 1.6–5.2% (0.09–0.32 µg Eq/g tissue) of the liver TRR. The parent chlorfenapyr and metabolites M-8, M-5 and M-4 constituted 6.5–30.9% (0.04–0.27 ppm), 0.4–1.9% (< 0.01–0.01 µg Eq/g tissue), 4.6–16.1% (0.02–0.16 µg Eq/g tissue) and 3.6–20.6% (0.02–0.21 µg Eq/g tissue) of the TRR in the muscle, respectively.

The chemical structures of the identified metabolites of chlorfenapyr in the rat are illustrated in Figure 2.

Figure 2. Chemical structures of identified metabolites of chlorfenapyr (CL 303,630) in the rat

M-1

M-2

M-4
CL 152,837

M-5
CL 325,195

M-6
CL 152,835

M-7
CL 152,834

M-7A
CL 325,157

M-8
CL 303,268

M-9
CL 303,630

In summary, orally administered chlorfenapyr excreted via the faeces consisted mainly of the unchanged compound plus minor amounts of *N*-dealkylated, debrominated and hydroxylated oxidation products. The absorbed residue was metabolized via *N*-dealkylation, dehalogenation, hydroxylation and conjugation. The unchanged compound and its less polar metabolites were found in tissues such as fat and liver, whereas more polar metabolites and conjugates were present in the urine and in the highly perfused tissues, such as kidney and liver. The bond between the phenyl and pyrrole rings appears to remain intact (Mallipudi, 1994).

In the study by Okada (1994a) described in section 1.1, 11 components were detected in urine, which accounted for more than 0.1% of the dose individually, of which U-9 was a major metabolite detected at the highest level in all the groups. It was followed by U-3, U-4, U-7 and U-8, which showed relatively high levels.

In faeces, as in urine, continuous elution was observed, including minor peaks; there were 25 components individually containing more than 0.1% of the dose. The peak showing the highest level was F-25, which proved to be unchanged chlorfenapyr. Other than this, F-6, F-9, F-12 and F-20 showed relatively high levels. Metabolites such as F-20 showing relatively longer retention times on HPLC columns were non-polar ones, which were not present in the urine.

Also in bile, a number of peaks were present, but polar metabolites eluting at the retention time of 10–20 minutes accounted for a far higher proportion than in the urine and faeces. The metabolites eluted in this range were mixtures with complex composition and showed subtle differences in elution pattern with different samples (they were likely to be conjugates of 2-(4-chlorophenyl)-5-hydroxy-4-oxo-5-(trifluoromethyl)-2-pyrrolidine-3-carbonitrile, or PY-4-CO-5-OH). Other than these polar metabolites, components similar to urinary and faecal metabolites, such as B-9, B-1 l and B-17, were detected.

Contents of total metabolites in the urine are shown in Table 10. After a peak (U-1) eluting at the solvent front, there was continuous elution of ^{14}C, with minor peaks, suggesting that chlorfenapyr undergoes complex metabolism.

Table 10. Summary of the total urinary metabolites collected through 72 hours, determined using HPLC with refractive index detector

Metabolite peak	Identification	Contents (% of dose)			
		2 mg/kg bw		20 mg/kg bw	
		Males	Females	Males	Females
U-1	—	0.2	0.1	0.1	—
U-2	Conjugated metabolite[a]	1.2	0.5	0.7	0.5
U-3	Conjugated metabolite[a]	1.4	0.9	1.3	0.8
U-4	Conjugated metabolite[a]	1.8	0.8	1.7	0.6
U-5	—	0.9	0.5	0.4	0.4
U-6	—	0.6	—	0.1	—
U-7 (F-9/B-9)	PY-4-CO-5-COOH-5-H	1.1	0.8	0.8	0.6
U-8	—	1.0	0.9	0.8	0.8
U-9 (F-12/B-11)	PY-4-CO-5-OH	2.7	2.8	2.3	2.7
U-10	PY-4-CO-5-H	0.1	—	0.1	—
U-11	PY-4-OH	0.6	0.3	0.4	0.3
Procedural loss	[SepPak®]	1.4	0.8	0.9	0.7
Total		13.0	8.4	9.6	7.4

From Okada (1994b)
—, not detected (< 0.1% of dose)
[a] Conjugates of PY-4-CO-5-OH and its further metabolized compounds.

Table 11 shows the contents of total metabolites in the faecal samples. As in the urine, there was continuous elution of minor peaks, with 25 individual components, each representing more than 0.1% of the dose. The highest concentration was peak F-25, which corresponded to unchanged chlorfenapyr. F-6, F-9, F-12 and F-20 were non-polar metabolites not present in the urine.

Table 11. Summary of the total faecal metabolites collected through 72 hours, determined using HPLC with refractive index detector

Metabolite peak	Identification	Contents (% of dose)			
		2 mg/kg bw		20 mg/kg bw	
		Males	Females	Males	Females
F-1	—	0.7	0.6	0.7	0.2
F-2	Conjugated metabolite[a]	0.8	1.2	0.7	0.9
F-3	Conjugated metabolite[a]	2.2	1.2	1.6	1.1
F-4	Conjugated metabolite[a]	1.0	0.7	0.3	0.7
F-5	Conjugated metabolite[a]	1.3	1.4	1.1	1.5
F-6	Conjugated metabolite[a]	8.5	7.3	6.3	4.3
F-7	—	1.9	1.3	—	1.5
F-8	—	2.1	1.2	0.7	0.9
F-9 (U-7/B-9)	PY-4-CO-5-COOH-5-H	1.4	2.5	2.2	2.4
F-10	—	—	—	0.1	0.4
F-11	—	—	—	0.1	—
F-12 (U-9/B-11)	PY-4-CO-5-OH	3.8	3.1	2.8	2.5
F-13	—	—	0.3	0.2	0.2
F-14	—	—	0.5	0.3	0.2
F-15	—	1.4	1.2	1.1	1.5
F-16	—	—	0.2	—	—
F-17	—	—	0.2	—	0.2
F-18	—	—	0.1	—	—
F-19	—	—	0.1	0.6	0.2
F-20 (B-17)	M-ω-COOH	1.6	1.4	0.9	1.1
F-21	—	—	0.1	—	—
F-22	PY	0.3	0.6	0.3	0.3
F-23	M-4-H	0.5	0.4	0.7	0.6
F-24	—	—	—	0.1	—
F-25	Chlorfenapyr	17.0	23.1	35.2	33.0
Procedural loss	[SepPak®]	4.2	5.2	6.3	6.5
Unextracted ^{14}C	—	19.0	16.5	16.9	17.0
Total		67.7	70.4	79.2	77.2

From Okada (1994b)

—, not detected (< 0.1% of dose)

[a] Conjugates of PY-4-CO-5-OH and its further metabolized compounds.

Bile samples contained polar metabolites (B-2 through B-6), which accounted for higher proportions than metabolites eluted at similar retention times in the urine or faeces (Table 12).

Table 12. Summary of the total biliary metabolites collected through 24 hours, determined using HPLC with refractive index detection

Metabolite peak	Identification	Contents (% of dose)			
		2 mg/kg bw		20 mg/kg bw	
		Males	Females	Males	Females
B-1	—	0.7	0.6	0.4	0.3
B-2	Conjugated metabolite[a]	4.3	3.4	2.8	3.3
B-3	Conjugated metabolite[a]	5.2	5.0	3.2	4.0
B-4	Conjugated metabolite[a]	2.3	1.2	1.1	1.0
B-5	Conjugated metabolite[a]	5.8	4.7	3.3	3.6
B-6	Conjugated metabolite[a]	3.1	2.6	1.9	2.1
B-7	—	0.5	—	—	—
B-8	—	0.3	0.3	0.2	0.2
B-9 (U-7/F-9)	PY-4-CO-5-COOH-5-H	1.2	0.8	0.7	0.7
B-10	—	—	0.2	—	—
B-11 (U-9/F-12)	PY-4-CO-5-OH	1.5	1.4	0.8	1.3
B-12	—	0.2	0.1	0.2	0.1
B-13	PY-4-CO-5-H	0.5	0.4	0.6	0.4
B-14	—	0.4	0.6	0.4	0.4
B-15	—	0.1	—	—	—
B-16	—	—	—	—	—
B-17 (F-20)	M-ω-COOH	0.2	—	0.1	0.1
Procedural loss	[SepPak®]	3.8	2.8	1.7	2.4
Total		30.1	24.1	17.4	19.9

From Okada (1994b)
—, not detected (< 0.1% of dose)
[a] Conjugates of PY-4-CO-5-OH and its further metabolized compounds.

In summary, the major metabolic pathway of absorbed chlorfenapyr was the formation of PY-4-CO-5-OH by *N*-dealkylation, followed by transformation at position 4 of the pyrrole ring. Other metabolites retaining the *N*-alkyl group included M-4-H and M-ω-COOH. All the metabolites that either were successfully identified or had their structure estimated retained both the pyrrole and phenyl rings, which suggested that the bond between the two rings was resistant to cleavage during the metabolic transformation of chlorfenapyr. There was evidence of reabsorption of biliary metabolites (Okada, 1994b), supporting the existence of enterohepatic circulation of chlorfenapyr, previously suggested in a separate report (refer to Okada, 1994a).

The proposed metabolic pathway of chlorfenapyr in rats (Okada, 1994b) is illustrated in Figure 3.

2. Toxicological studies

2.1 Acute toxicity

Acute toxicity studies on chlorfenapyr are summarized in Table 13.

Figure 3. Proposed metabolic pathway of chlorfenapyr in rats

[PY]
[PY-4-OH]
[PY-4-CO-5-H]
[PY-4-CO-5-OH]
Polar Metabolites
[M-ω-COOH]
MK-242
[M-4-H]
[PY-4-CO-5-COOH-5-H]

Table 13. Summary of acute toxicity, including irritancy and skin sensitization, of chlorfenapyr

Type of test	Result	Purity; batch no.	Reference
Mouse, oral	LD_{50} = 45 mg/kg bw (males) LD_{50} = 78 mg/kg bw (females)	94.5%; AC7504-59A	Bradley (1994a)
Rat, oral	LD_{50} = 441 mg/kg bw (males) LD_{50} = 1152 mg/kg bw (females)	94.5%; AC7504-59A	Lowe (1993)
Rabbit, dermal	LD_{50} > 2000 mg/kg bw	94.5%; AC7504-59A	Fischer (1992)
Rat, 4 h inhalation	LC_{50} = 0.83 mg/l (males)	94.5%; AC7504-59A	Hoffman (1993)
Rabbit, skin irritation	Not irritating	94.5%; AC7504-59A	Bradley (1993a)
Rabbit, eye irritation	Not irritating	94.5%; AC7504-59A	Bradley (1993b)
Guinea-pig, skin sensitization (Magnusson & Kligman)	Not sensitizing	94.5%; AC7504-59A	Otake (1995)

LC_{50}, median lethal concentration; LD_{50}, median lethal dose

(a) Lethal doses

Oral administration

In a test for acute oral toxicity, five mice (CRL:CD®BR) of each sex per dose received chlorfenapyr (BAS 306 I) (lot no. AC 7504-59A; purity 94.5%) in 0.5% carboxymethylcellulose and tap water by oral gavage at a dose level of 35, 70 or 140 mg/kg bw (dose volume 25 ml/kg bw) following a preliminary range-finding study. The study was conducted in compliance with United States Environmental Protection Agency (USEPA) test guideline OPPTS 870.1100_EPA 81.1. Overt signs of toxicity were observed at the 140 mg/kg bw dose level and were limited to decreased activity during the first 2 hours following dosing. Mortality occurred in both sexes at all levels of dosing and generally occurred during the first 24 hours following dosing. No gross pathological changes in surviving mice could be attributed to ingestion of the test material. Gross pathological changes in decedents were limited to bright red lungs in one mouse at 140 mg/kg bw. There were no consistent necropsy findings associated with active ingredient administration.

The median lethal dose (LD_{50}) was 45 mg/kg bw for males and 78 mg/kg bw for females (Bradley, 1994a).

The acute oral LD_{50} of chlorfenapyr (lot no. AC 7504-59A; purity 94.5%) was evaluated by oral gavage in 0.5% weight per volume (w/v) carboxymethylcellulose and tap water in five male rats (CRL:CD(SD)®BR) per dose group at a dose level of 156.25, 312.5, 625, 1250 or 2500 mg/kg bw and five female rats (CRL:CD(SD)®BR) per dose group at a dose level of 625, 1250 or 2500 mg/kg bw. In both cases, chlorfenapyr was administered at a constant dose volume of 10 ml/kg bw. The study was conducted partially in compliance with USEPA test guideline OPPTS 870.1100_EPA 81.1. Mortalities were observed in a dose-dependent manner in both sexes, starting from a dose level of 312.5 mg/kg bw in males and from a dose level of 625 mg/kg bw in females.

Signs of toxicity included hyperthermia and prostration in both sexes at dose levels of 625 mg/kg bw and above and prostration and salivation in males at dose levels of 312.5 mg/kg bw and above. Signs of hyperthermia included warm to the touch and an elevation of rectal body temperature, which was observed between 15 minutes and 24 hours after dosing. The majority of clinical signs were resolved in surviving animals by 24 hours after treatment. Gross pathological findings, such as muscle tetany, abdominal muscle striation, and congested, pale and mottled liver and kidney, were observed in both sexes at dose levels of 625 mg/kg bw and above and in males also at a dose level of 312.5 mg/kg bw.

The LD_{50} of chlorfenapyr was calculated to be 441 and 1152 mg/kg bw for males and females, respectively (Lowe, 1993).

Dermal application

In a test for acute dermal toxicity, five male and five female rabbits (New Zealand White strain) were exposed to chlorfenapyr (lot no. AC 7504-59A; purity 94.5%) moistened with tap water at a dose level of 2000 mg/kg bw by dermal occlusive application to intact skin for a 24-hour period. The application site encompassed an area equivalent to approximately 10% of the body surface area. The protocol was in compliance with USEPA test guideline OPPTS 870.1200_EPA 81.1. One female died on day 2 of the study. Prior to death, no overt signs of toxicity were observed. At necropsy, the animal exhibited pale kidneys, a pale spleen, mottled lungs and evidence of haemorrhage externally in the anogenital region. This haemorrhage was possibly caused by the occlusive wrapping during the 24-hour exposure period. The death did not appear to be treatment related. No overt signs of toxicity were observed. Body weights and body weight gains were generally unaffected in surviving animals. No treatment-related gross lesions were observed in surviving animals at termination of the 14-day observation period. The dermal LD_{50} in male and female rabbits was greater than 2000 mg/kg bw (Fischer, 1992).

Exposure by inhalation

In a whole-body inhalation study, five rats (Sprague-Dawley CDR) of each sex per dose group were exposed to a mean analytical concentration of 0.34, 0.71, 1.8 or 2.7 mg/l (by gravimetry) of chlorfenapyr (lot no. AC 7504-59A; purity 94.5%) (dust) for 4 hours. An additional five rats of each sex served as controls. The study protocol was partially in compliance with test method B.2 of Directive 92/69/EEC and Organisation for Economic Co-operation and Development (OECD) Test Guideline No. 403, Acute Inhalation Toxicity (1981). The mean analytical concentrations along with the mass median aerodynamic diameter (MMAD) and the corresponding geometric standard deviation (GSD) are summarized in Table 14.

Table 14. Acute inhalation toxicity of chlorfenapyr in rat: particle size data

Group	Nominal concentration (mg/l)	Analytical concentration (mg/l)	Mortality			MMAD (µm)	GSD
			Male	Female	Total		
I	—	0	0/5	0/5	0/10	—	—
II	17	1.8	4/5	1/5	5/10	7.3	2.0
III	5.8	0.71	2/5	0/5	2/10	7.0	1.9
IV	3.9	0.34	1/5	0/5	1/10	8.1	2.3
V	80	2.7	5/5	1/5	6/10	5.9	1.9

From Hoffman (1993)

Fourteen animals died before the end of the study. All remaining animals were examined post mortem for the presence of grossly visible abnormalities. Among toxicological findings, only discoloration of the lungs and tan/brown skin discoloration were believed to be treatment related. The median lethal concentration (LC_{50}), based on the analytical concentration, was calculated to be 1.9 mg/l for the combined sexes, 0.83 mg/l for the males and greater than 2.7 mg/l for the females.

The study (Hoffman, 1993) has the following weak points:

- The differences between the nominal and analytical exposure concentrations were attributed to impaction or sedimentation of the dust on the surface of the exposure chamber. In this context, although a whole-body exposure is foreseen by the guideline used, no precaution was taken to prevent licking of the test substance from the skin.
- The mean MMAD was calculated to be 7.1 µm with a GSD of 2.
- The results demonstrate that approximately 32% of the particles were less than 5 µm in size and 71% of the particles were less than or equal to 10 µm in size.

(b) Dermal irritation

The skin irritation potential of chlorfenapyr (lot no. AC 7504-59A; purity 94.5%) was tested in three male albino rabbits (New Zealand White strain). The study was conducted according to test method B.4 of Directive 92/69/EEC and OECD Test Guideline No. 404, Acute Dermal Irritation/Corrosion (1992). The animals were exposed to 0.5 g chlorfenapyr moistened with tap water, applied to a 6.5 cm^2 gauze pad and applied to trunk skin under an occlusive cover for 4 hours. A barely perceptible erythema was observed in two of the three test animals at the 1-hour observation. At the 24-hour observation, all signs of skin irritation had resolved. No further signs of irritation were observed at 24, 48 or 72 hours after removal of the test material. Chlorfenapyr was not irritating to rabbit skin (Bradley, 1993a).

(c) Ocular irritation

The eye irritation potential of chlorfenapyr was investigated in three male rabbits (New Zealand White albino) by instillation of 0.1 g into the conjunctival sac of the left eye. Eyes were examined at −6, 1, 24, 48 and 72 hours and at 4 days. The study was conducted in accordance with test method B.5 of Directive 92/69/EEC and OECD Test Guideline No. 405, Acute Eye Irritation/Corrosion (1987). Eye irritation at 1 hour after treatment was characterized by slight redness of the conjunctiva, slight chemosis and slight discharge in all three animals. At 24 hours post-dosing, eye irritation was characterized by a diffuse area of corneal opacity (1/3), slight iritis (1/3), slight (2/3) to moderate (1/3) conjunctival redness and slight (1/3) to moderate (1/3) chemosis. At 48 hours post-dosing, corneal opacities were unchanged, the iritis had resolved and conjunctival irritation was limited to slight redness (3/3). At 72 hours post-dosing, eye irritation was limited to slight redness of the conjunctiva in one animal, while all signs of irritation had resolved in the remaining test animals. All signs of irritation had resolved in the remaining animal by 4 days post-dosing. Chlorfenapyr is considered to be practically non-irritating to the eye in this test (Bradley, 1993b).

(d) Dermal sensitization

The skin sensitization potential of chlorfenapyr (lot no. AC 7504-59A; purity 94.5%) was investigated using topical and intradermal (maximization) methods in groups of 40 (control and test) female Hartley guinea-pigs. The protocol was partly in compliance with test method B.6 of Directive 92/69/EEC and OECD Test Guideline No. 406, Skin Sensitisation (1992). Chlorfenapyr was dissolved in olive oil. Test concentrations were selected on the basis of preliminary testing and were 2% for intradermal injection, 10% by occlusive application over 48 hours for dermal induction (seen to be moderately irritating) and 0.4% for application for 24 hours for challenge (highest non-irritant concentration). No dermal reaction was reported in the test substance groups. Therefore, chlorfenapyr was concluded not to be sensitizing in this study (Otake, 1995).

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

Chlorfenapyr technical (lot no. AC6943-61A; purity 98.4%) was fed to groups of five male and five female CD-1 albino mice for 28 days at a dietary concentration of 0 (control), 160, 240, 320, 480 or 640 ppm (equal to 0, 30.1, 43.6, 62.3, 100.5 and 143.6 mg/kg bw per day for males and 0, 33.7, 57.8, 71.1 and 122.6 mg/kg bw per day for females, respectively; dose not calculated for 640 ppm females, as it caused death in all females by day 5). The protocol was partially in compliance with test method B.7 of Directive 92/69/EEC and OECD Test Guideline No. 407, Repeated Dose 28-Day Oral Toxicity Study in Rodents (1981). Deviation from the protocol was due to the absence of blood biochemistry analysis.

Mortality (Table 15) occurred in male mice at the 640 ppm (4/5), 480 ppm (1/5) and 240 ppm (1/5) dietary levels and in female mice at the 640 ppm (5/5), 480 ppm (3/5) and 320 ppm (1/5) levels. All mortality occurred during the first 7 days of the study. Signs of toxicity were observed in both sexes at the 640 and 480 ppm levels and in females at the 320 ppm level. Signs of toxicity observed included ataxia, depression, stiff gait and body drop.

Dose-related decreases in body weight gain (Table 15) were noted at all dietary levels, although these were not statistically significant. Body weight gains were reduced in the surviving male at 640 ppm (41%), in surviving males and females at 480 ppm and 320 ppm (combined average of 62% and 27%, respectively) and in males at 240 ppm (30%). Weight gains for both sexes were slightly reduced at 160 ppm (average of 21%).

Statistically significant haematological changes (Table 15) observed at termination consisted of decreased lymphocyte counts and increased eosinophil counts in surviving females at 480 ppm when compared with controls. The total white blood cell count for females at 480 ppm was

Table 15. Oral 28-day toxicity of chlorfenapyr in mouse

	0 ppm		160 ppm		240 ppm		320 ppm		480 ppm		640 ppm	
	M	F	M	F	M	F	M	F	M	F	M	F[a]
Mortality	0/5	0/5	0/5	0/5	1/5	0/5	0/5	1/5	1/5	3/5	4/5	5/5
Body weight (g) (week 4)	36.6	28.8	35.8	27.2	34.6	28.8	34.8	26.9	32.4*	25.7	33.5	—
	(—)	(—)	(↓2%)[b]	(↓5.6%)	(↓5.5%)	(0%)	(↓5%)	(↓6.6%)	(↓11.5%*)	(↓10.8%)	(↓9.5%)	(—)
Body weight total gain (g)[c]	7.6	4.6	6.4	3.4	5.3	4.6	5.8	3.2	3.8	1.2	4.4	—
	(—)	(—)	(↓15.8%)[b]	(↓26%)	(↓30%)	(0%)	(↓23.7%)	(↓30%)	(↓50%)	(↓74%)	(↓41%)	(—)
Organ weights												
Absolute liver weight (g)	1.4	1.2	1.6	1.19	1.6	1.4*	1.6	1.3	1.7	1.4	2	—
Relative liver weight (% change relative to control)	—	—	↑9.6%	↓4.1%	↑13%	↑8.6%	↑14%	↑9%	↑30%*	↑23%*	↑47%	
Haematology												
Lymphocytes (%)	66.8	81	67.8	78	62.5	79.8	61.2	77.8	72.8	63.5*	84	—
Eosinophils (%)	—	—	0	0.2	0.3	0.8	0	0.8	0	6*	0	—

From Fischer (1991b)

* $P < 0.05$ (Williams' test)

[a] No parameters except mortality could be assessed in females at 640 ppm.

[b] Per cent change relative to controls.

[c] Body weight gain in grams during weeks 1–4.

comparable to that of controls. It should be noted that these results are based on only two surviving females (out of five) in the 480 ppm group. The increase in eosinophil counts was not considered biologically significant because eosinophil counts for males at 480 and 640 ppm (the highest concentration tested) were comparable to those of controls. Similarly, lymphocyte counts for males at 480 ppm were comparable to those of controls, and a slight, but not statistically significant, increase in lymphocyte counts was observed for males at 640 ppm. As changes in lymphocyte counts noted for females in the 480 ppm group (highest concentration at study termination) were based on observations in only two animals and were not observed in males at a higher concentration (640 ppm), they were not considered to be biologically significant.

Increased absolute and relative liver weights (Table 15) were observed in both sexes at dose levels of 240 ppm and higher and in males also at the 160 ppm level, although they were statistically significant only at 480 ppm. It was noted that the relative liver weight in one of the control males was somewhat low. These increases were accompanied by hepatocellular hypertrophy, which was observed microscopically at all treatment levels.

In conclusion, the no-observed-adverse-effect level (NOAEL) for chlorfenapyr in the mouse was 160 ppm (equal to 30.1 mg/kg bw per day in males and 33.7 mg/kg bw per day in females), based on decreased body weight gain, mortality and increased relative liver weight seen at 240 ppm (equal to 43.6 mg/kg bw per day in males and 57.8 mg/kg bw per day in females) (Fischer, 1991b).

Chlorfenapyr (lot no. AC 7171-141A; purity 93.6%) was fed to five groups of 20 male and 20 female albino CD-1 mice for 13 weeks at a dietary level of 0 (control), 40, 80, 160 or 320 ppm (equal to 0, 7.1, 14.8, 27.6 and 62.6 mg/kg bw per day for males and 0, 9.2, 19.3, 40.0 and 78.0 mg/kg bw per day for females, respectively).

Two mice at the 320 ppm level died during the study (Table 16). These deaths were attributed to ingestion of chlorfenapyr. Clinical signs of toxicity, consisting of diuresis and tremors, were observed in one male at the 320 ppm level from day 14 through day 19 of the study. No other signs of toxicity could be attributed to the test material at any other dietary level during the study period. Feed consumption at all dietary levels was comparable to that of controls for most of the measurement intervals. Treatment-related reductions in body weight gain were noted for both sexes at the 320 ppm level and for females at the 160 ppm level when compared with control values.

Haematological changes noted at the 320 ppm level included statistically significant increases in haematocrit and erythrocyte counts in males and a statistically significant increase in white blood cell counts in females. Changes in clinical chemistry parameters noted at the 320 ppm level included increased sodium and decreased albumin in males and increased potassium in females. Dose-dependent, statistically significant increases in liver to body weight ratios were observed in both sexes at the 320 ppm level and in males at the 160 ppm level. Spleen to body weight ratios were also significantly increased in male mice at the 320 and 160 ppm dietary levels. The percentage change was very similar at the two dose levels.

No treatment-related macroscopic changes were noted at termination. Microscopic evaluation revealed treatment-related hypertrophy of liver parenchymal cells in male mice at the 320 ppm (19/20 males; 10/20 females), 160 ppm (13/20 males; 4/20 females), 80 ppm (6/20 males; 0/20 females) and 40 ppm (1/20 males; 0/20 females) dietary levels, compared with controls (0/20 males; 0/20 females). There was no evidence of progressive degenerative change, toxic necrosis or proliferative change. Male and female mice at the 320 ppm level exhibited spongiform (encephalo)myelopathies in the brain (19/20 males; 19/20 females) and spinal cord (18/20 males; 19/20 females). One male at the 160 ppm level also exhibited myelopathy in the spinal cord.

Table 16. Oral 90-day toxicity of chlorfenapyr in mouse

	0 ppm		40 ppm		80 ppm		160 ppm		320 ppm	
	M	F	M	F	M	F	M	F	M	F
Mortality	0/20	1/20	0/20	0/20	0/20	0/20	0/20	0/20	1/20	1/20
Body weight (g) (week 13)	41	34.1	39.9	34.3	39.9	33.6	40.6	32.5	38*	31.6*
	(—)	(—)	(↓2.7%)[a]	(↑0.6%)	(↓2.7%)	(↓1.5%)	(↓1.0%)	(↓4.7%)	(↓7.3%*)	(↓7.3%*)
Body weight total gain (g)[b]	11.8	9.8	10.4	9.7	10.2	9.6	11.9	8.1	8.7*	7*
	(—)	(—)	(↓11.9%)[a]	(↓1.0%)	(↓1.9%)	(↓2.0%)	(↑0.8%)	(↓17.3%)	(↓26.3%*)	(↓28.6%*)
Organ weights										
Absolute liver (g)	2.46	2.04	2.54	2.17	2.46	2	2.64	2	2.63	2.21
Relative liver (% change relative to control)	—	—	↑5.0%	↑6.2%	↑2.0%	↑3.6%	↑10.3%*	↑5.7%	↑13.3%*	↑18.0%*
Absolute spleen (g)	0.12	0.16	0.13	0.16	0.14	0.15	0.15*	0.15	0.15	0.16
Relative spleen (% change relative to control)	—	—	↑6.5%	↑4.4%	↑12.9%	0%	↑25.2%*	↑2.2%	↑25.5%*	↑15.6%
Blood chemistry										
Sodium (week 13, % change relative to control)	—	—	↑1.0%	↑0.2%	↓0.1%	↓0.6%	↑0.5%	↑1.9%	↑1.4%*	↑4.1%
Potassium (week 13, % change relative to control)	—	—	↑4.9%	−2.5%	0%	−2.5%	0%	↑11.3%	[c]	↑32.5%*
Total protein (week 13, % change relative to control)	—	—	↑1.9%	↑5.6%	↑1.9%	0%	↑3.8%	↑3.7%	↑1.9%	↑9.3%*
Albumin (week 13, % change relative to control)	—	—	0%	0%	↓4.0%	↓6.9%	↓8.0%	↓6.9%*	↓12.0%*	↓6.9%
Haematology										
Haematocrit (% change relative to control)	—	—	↑2.6%	↓2.4%	↑1.9%	↑3.6%	↑1.0%	↓2.6%	↑17.9%*	↑8.4%
RBC (% change relative to control)	—	—	↑2.5%	↓6.1%	↑1.3%	↓3.7%	↑2.5%	↓6.1%	↑11.4%*	↑1.2%
Lymphocyte (% change relative to control)	—	—	↑2.6%	↓1.1%	↑14.0%*	↓2.2%	↑12.1%*	↓4.7%	↑10.5%	↓3.0%
WBC (% change relative to control)	—	—	↑85.0%	↓12.9%	↑85.0%	↑9.7%	↑25.0%	↓25.8%	↑60.0%	↑109.7%*
Neutrophyle (% change relative to control)	—	—	↓5.3%	↑5.3%	↓31.2%*	↑8.4%	↓27.9%*	↑21.1%	↓22.6%	↑6.3%
Histopathology (n = 20)										
Myelopathies of the brain	0	0	0	0	0	0	0	0	19	19
Myelopathies of the spinal cord	0	0	0	0	0	0	1	0	18	19

From Fischer (1994)

F, female; M, male; RBC, red blood cells; WBC, white blood cells

* $P < 0.05$ (Williams' test)

[a] Per cent change relative to controls.

[b] Body weight gain in grams during weeks 1–13.

[c] No evaluation done, sample exhibited haemolysis.

The NOAEL for chlorfenapyr in the mouse was 80 ppm (equal to 14.8 mg/kg bw per day in males and 19.3 mg/kg bw per day in females), based on increased relative spleen weight and myelopathy in brain and spinal cord in male mice seen at 160 ppm (equal to 27.6 mg/kg bw per day). Hepatic hypertrophy observed in males in the absence of any other histopathological correlate was not considered adverse (Fischer, 1994).

Rats

Chlorfenapyr technical (lot no. AC 6943-61A; purity 98.4%) was fed to six groups of five male and five female Sprague-Dawley albino rats for 28 days at a dietary concentration of 0 (control), 600, 900, 1200, 1600 or 2000 ppm (equal to 0, 68.3, 106.3, 134.2, 176.8 and 243.0 mg/kg bw per day in males and 0, 74.8, 110.5, 147.2, 192.7 and 247.9 mg/kg bw per day in females, respectively). The selection of dose levels was based on a preliminary study. The protocol was in compliance with test method B.7 of Directive 92/69/EEC and OECD Test Guideline No. 407, Repeated Dose 28-Day Oral Toxicity Study in Rodents (1981).

Mortality (Table 17) occurred in males at the 2000 ppm (2/5) and 1600 ppm (1/5) dietary levels. All females survived until study termination. No overt signs of toxicity that could be attributed to administration of the test material were observed in the surviving animals during the study.

Dose-related, statistically significant decreases in feed consumption and body weight gain were noted at 1200–2000 ppm. Reductions in body weight gain occurred in both males and females and averaged 42% at 2000 ppm, 28% at 1600 ppm, 16% at 1200 ppm, 12.5% at 900 ppm and 6% at 600 ppm. Liver weights (absolute and relative to body weight) were increased in all dose groups and were dose dependent. Relative kidney weight was dose dependently increased in males from 900 ppm.

Haematological parameters were unaffected by treatment with chlorfenapyr. Values for alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), blood urea nitrogen and total protein were somewhat increased over control values in both male and female rats at the 2000, 1600 and 1200 ppm dietary levels.

Hepatocellular hypertrophy was observed in all surviving animals (3/3 males and 5/5 females) at 2000 ppm and in 75% (3/4) of surviving males and 40% (2/5) of surviving females at 1600 ppm.

In conclusion, the NOAEL for chlorfenapyr in rats was 600 ppm (equal to 68.3 mg/kg bw per day in males and 74.8 mg/kg bw per day in females), based on a relative liver weight increase (> 20% of control in both sexes) and ALT increase at 900 ppm (equal to 106.3 mg/kg bw per day in males and 110.5 mg/kg bw per day in females) (Fischer, 1991a).

Chlorfenapyr technical (lot no. AC 7171-141A; purity 93.6%) was fed to six groups of 20 male and 20 female albino rats for 13 weeks at a dietary concentration of 0 (control), 150, 300, 600, 900 or 1200 ppm (equal to 0, 10.9, 22.0, 44.9, 69.5 and 92.2 mg/kg bw per day in males and 0, 12.5, 26.1, 51.8, 75.4 and 102.8 mg/kg bw per day in females). The protocol was in compliance with test method B.7 of Directive 92/69/EEC and OECD Test Guideline No. 408, Repeated Dose 90-Day Oral Toxicity Study in Rodents (1981).

No treatment-related mortality was noted at any dose level (Table 18). Low incidences of clinical signs, including ataxia (3/20), decreased activity (3/20), dark material around nose (4/20) and anorexia (5/20), were observed in a few males of the 1200 ppm group. An increased incidence of brown material around the nose was also observed in males at 1200 ppm (9/20).

Body weights and body weight gains were lower than control values for both sexes at 1200 and 900 ppm and for males only at 600 ppm. Total body weight gains were decreased in males and females by 37% and 24%, respectively, at 1200 ppm, by 25% and 21%, respectively, at 900 ppm and by 14% and 8%, respectively, at 600 ppm.

Table 17. Oral 28-day toxicity of chlorfenapyr in rat

	0 ppm		600 ppm		900 ppm		1200 ppm		1600 ppm		2000 ppm	
	M	F	M	F	M	F	M	F	M	F	M	F
Mortality	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	2/5	0/5
Body weight (g) (week 4)	312 (—)	228 (—)	304 (↓2.6%)[a]	217 (↓4.9%)	280 (↓10.3%)	215 (↓5.7%)	277 (↓11.3%)	205* (↓10%*)	248* (↓20.6%*)	189* (↓17.1%*)	219* (↓30%*)	174* (↓23.7%*)
Body weight total gain (g)[b]	212 (—)	145 (—)	204 (↓3.8%)[a]	134 (↓7.6%)	179.4 (↓15%)	131.8 (↓10%)	176.8 (↓17%)	122.4* (↓15.6*)	149* (↓30%*)	107* (↓26%*)	113.7* (↓46%*)	92.2* (↓37%*)
Organ weights												
Absolute liver (g)	10.2	7.4	10.6	8.7	11	9*	11.5	9.3*	12.5*	9.9*	11.8	10.2*
Relative liver (%)	3.6	3.6	3.9	4.4*	4.4*	4.6*	4.6*	5*	5.6*	5.7*	6.1*	6.5*
Absolute kidney (g)	2.4	1.8	2.1	1.7	2	1.7	2	1.6*	1.6*	1.5*	1.6*	1.4*
Relative kidney (%)	0.8	0.9	0.8	0.9	0.8	0.9	0.8	0.9	0.7	0.9	0.8	0.9
Blood chemistry												
BUN (% change relative to control)	—	—	↑13.3%	↑26.8%	↑17.3%	↑19.7%	↑28.0%	↑35%*	↑48%*	↑42%*	↑71%*	↑56%*
Albumin (% change relative to control)	—	—	↓7.3%	↓5.1%	↓12.2%	↓7.7%	↓12%*	↓5.1%	↓15%*	↓10%*	↓17%*	↓10%*
Total protein (% change relative to control)	—	—	↓2.7%	↑6.0%	↓4.1%	↑6.0%	↓1.4%	↑9%*	↑4.1%	↑9%*	↓4.1%	↑10%*
ALT (% change relative to control)	—	—	↓10.3%	↑22.2%	↑6.1%	↑67.0%	↑25.7%	↑36.2%	↑43%*	↑79%*	↑71%*	↑98%*
GGT (U/l)	0	0.6	0	0.8	0.2	1	0.6	1.4	2.8*	2	4.7*	5.4*

From Fischer (1991a)

ALT, alanine aminotransferase; BUN, blood urea nitrogen; F, female; GGT, gamma-glutamyl transpeptidase; M, male; U, units; * $P < 0.05$ (Williams' test)

[a] Per cent change relative to controls.

[b] Body weight gain in grams during weeks 1–4.

Table 18. Oral 90-day toxicity of chlorfenapyr in rat

	0 ppm		150 ppm		300 ppm		600 ppm		900 ppm		1200 ppm	
	M	F	M	F	M	F	M	F	M	F	M	F
Mortality	0/20	1/20	0/20	0/20	0/20	0/20	0/20	1/20	0/20	0/20	0/20	0/20
Body weight (g) (week 13)	531	313.4	502	324.2	501	311.5	478.3*	299.8	441.2*	274.4*	390.7*	270.3*
	(—)	(—)	(↓5.5%)[a]	(↓3.4%)	(↓5.6%)	(↓0.7%)	(↓10%*)	(↓4.4%)	(↓17%*)	(↓12.5%*)	(↓26.5%*)	(↓13.8%*)
Body weight total gain (g)[b]	376.2	188.9	347.1	192.4	347.7	184.1	323.4*	174.5	283.6*	148.5*	237.1*	143.9*
	(—)	(—)	(↓8%)[a]	(↑2%)	(↓8%)	(↓3%)	(↓14%*)	(↓8%)	(↓25%*)	(↓21%*)	(↓37%*)	(↓24%*)
Organ weights												
Absolute liver (g)	13.8	8.19	14.2	8.51	14.9	8.74	15	9.58	16*	9.7	14.9	10.31
Relative liver (% change relative to control)	—	—	↑7.2%	0%	↑13%*	↑6.7%	↑19.5%*	↑21.7%*	↑38%*	↑33%*	↑45%*	↑42.3%*
Absolute kidney (g)	3.31	2.26	3.46	2.28	3.34	2.23	3.21	2.24	2.99*	2*	2.69*	1.99*
Relative kidney (% change relative to control)	—	—	↑9%	↓1.3%	↑6%	0%	↑6%	↑3.8%	↑7.5%	↑1.2%	↑10.6%	↑1.2%
Absolute spleen (g)	0.8	0.58	0.8	0.59	0.83	0.61	0.83	0.61	0.97*	0.68*	0.95*	0.71*
Relative spleen (% change relative to control)	—	—	0%	↓6%	↑6.2%	↑4.4%	↑12.5%	↑9.9%	↑43%*	↑30%*	↑62.5%	↑40%*
Blood chemistry												
BUN												
- week 6 (% change relative to control)	—	—	↑5.6%	↑3.5%	↑19.7%	↑7.9%	↑18.3%	↑24.4%	↑24.6%	↑17.2%	↑57.7%*	↑37.4%*
- week 13 (% change relative to control)	—	—	↓6.5%	↓4.5%	↑2.3%	↓10%	↑4.6%	↓3.2%	↑8.7%	↓5.1%	↑30%*	↑5.7%
Total protein												
- week 13 (% change relative to control)	—	—	↑4%	↑3.8%	↑4%	0%	↑4%	0%	↑6.6%*	↑7.7%*	↑1.3%	↑7.7%*
Albumin												
- week 6 (% change relative to control)	—	—	0%	↓5.5%	0%	↓8.2%	↓2.8%	↓5.5%	↓2.8%	↓2.8%	↓5.6%*	↓5.5%

	0 ppm		150 ppm		300 ppm		600 ppm		900 ppm		1200 ppm	
	M	F	M	F	M	F	M	F	M	F	M	F
- week 13 (% change relative to control)	—	—	0%	0%	↑2.4%	↓5%	↓2.5%	↓5%	↓2.5%	↓2.4%	↓10%*	↓5%
ALP												
- week 6 (% change relative to control)	—	—	↓18.2%	↓5.9%	↓5%	↑25%	↑5%	↑18.4%	↑44%*	↑14.4%	↑21%	↑44.6%
- week 13 (% change relative to control)	—	—	↓3%	↓7%	↑14%	↑35%	↑30%	↑23%	↑111%*	↑72%*	↑104%*	↑102%*
Haematology												
Haematocrit (% change relative to control)	—	—	↑1%	↑0.2%	↓3%	↑0.5%	↓2.4%	↓6%	↓4.5%	↓7.3%*	↓7.5%*	↓9%*
Haemoglobin (% change relative to control)	—	—	1.3	↓0.6	↓3.8	↓1.3	↓3.8	↓6.9*	↓5.7	↓9.4*	↓7.0*	↓8.8*
RBC (% change relative to control)	—	—	↑2.4%	0%	↓3.6%	0%	↓2.4%	↓6.2%	↓3.6%	↓8.6%*	↓10.8%*	↓8.6%*
Platelets (% change relative to control)	—	—	↑5.8%	↓2.8%	↑4.1%	↑9.4%	↑11.3%	↑12.1%	↑21.1%*	↑14.1%	↑20.0%*	↑13.8%*
Histopathology (*n* = 20)												
Vacuolation of brain and spinal cord	0	0	0	0	0	0	1	0	2	0	2	0
Vacuolation of sciatic nerve	0	0	0	0	0	0	0	0	0	0	1	0
Vacuolation of optic nerve	0	0	0	0	0	0	0	0	0	0	1	0

From Fischer (1993)

ALP, alkaline aminotransferase; BUN, blood urea nitrogen; F, female; M, male; RBC, red blood cells; * $P < 0.05$ (Williams' test)

[a] Per cent change relative to controls.

[b] Body weight gain in grams during weeks 1–13.

Red blood cell parameters (haematocrit, erythrocyte count and haemoglobin) were reduced for both sexes at 1200 ppm and for females at 900 ppm; in females, a significant reduction of haemoglobin was also evident at the 600 ppm dose level. Haematocrit and erythrocyte count were reduced in this group, but not significantly. Platelet counts were elevated in both sexes at 1200 and 900 ppm, but the changes were statistically significant only in males. Serum alkaline phosphatase level tended to be higher after 6 weeks and was clearly elevated in both sexes at 1200 and 900 ppm after 13 weeks of treatment. GGT level in both sexes and ALT level in males were elevated at 1200 ppm after 6 weeks, but not 13 weeks. Blood urea nitrogen was elevated at 1200 ppm in both sexes after 6 weeks, but only in males after 13 weeks. Also after 13 weeks, total serum protein was elevated in females at 1200 ppm and in both sexes at 900 ppm. Albumin was reduced after 6 and 13 weeks in males at 1200 ppm.

Increased absolute and relative liver and spleen weights (correlated with decreased red cell parameters) were observed at termination in the 1200 and 900 ppm groups. Other organ weight changes at these treatment levels were secondary to body weight decreases.

Hepatic parenchymal cell hypertrophy was observed in one male at 1200 ppm and one male at 900 ppm. Spongiform myelopathy (vacuolation) of the brain and spinal cord was seen in two males at 1200 ppm, two males at 900 ppm and one male at 600 ppm. In the two males at 1200 ppm with myelopathies of the brain and spinal cord, one also exhibited this lesion in the sciatic nerve and the other in the optic nerve. Testicular atrophy was observed in two males at 1200 ppm and three males at 900 ppm.

The NOAEL for chlorfenapyr in the rat was 300 ppm (equal to 22.0 mg/kg bw per day in males and 26.1 mg/kg bw per day in females), based on increases in relative liver weight, alkaline phosphatase and blood urea nitrogen and, in females, changes in red cell parameters seen at 600 ppm (equal to 44.9 mg/kg bw per day in males and 51.8 mg/kg bw per day in females) (Fischer, 1993).

Dogs

Chlorfenapyr technical (lot no. AC 7504-59A; purity 95.4%) was fed to four groups of four male and four female purebred Beagle dogs for 90 days at a dietary concentration of 0 (control), 60, 120 or 300 ppm. These concentrations were based on a range-finding study in which dogs exhibited body weight losses at 300 ppm and above and reductions in feed consumption within short feeding intervals (less than 2 weeks) at 400 ppm and above. A stability study conducted at room temperature on 60 and 300 ppm mock batches of chlorfenapyr in Certified Canine Diet Meal 5007 demonstrated that these batches were stable for a period of 14 days. Diets were assayed weekly for the first 4 weeks and monthly thereafter. The batches resulted in mean nominal percentages of 95.4%, 95.1% and 97.2% for the three treatment groups, respectively. Dietary concentrations were 60, 120 and 300 ppm; due to poor feed consumption (and subsequent body weight losses and/or emaciated appearance in a few dogs) in the high-dose group, the dose level was decreased on test day 15 to 240 ppm and on test day 26 to 200 ppm. Substance intake was as follows: 2.1, 3.9, 4.4, 6.0 and 7.3 mg/kg bw per day for males and 2.2, 4.5, 6.0, 5.8 and 7.1 mg/kg bw per day for females.

Reduction of the dietary concentration of the high-dose group to 200 ppm was well tolerated, with feed consumption and body weight gain paralleling that of the control group. There were no effects on feed consumption or body weight gain in the 120 or 60 ppm groups (Table 19).

No treatment-related changes in ophthalmology, haematology, clinical chemistry, urine analysis, organ weight or pathology were evident at any dose level.

Based on these results, the NOAEL was judged to be 120 ppm (equal to 3.9 mg/kg bw per day in males and 4.5 mg/kg bw per day in females), based on decreased body gain at 200 ppm (equal to 7.3 mg/kg bw per day in males and 7.1 mg/kg bw per day in females) (Kelly, 1993).

Table 19. Oral 90-day toxicity of chlorfenapyr in dog: mean body weight changes in selected weeks

Week	Body weight change (kg)							
	0 ppm		60 ppm		120 ppm		200 ppm[a]	
	M	F	M	F	M	F	M	F
1	0.10	0.00	0.50	0.40	0.10	0.40	−1.10	−0.1
2	0.00	0.00	0.40	0.20	0.10	0.10	−1.60	−1.00
4	0.40	0.70	0.40	0.60	0.30	0.50	−1.3**	−0.9**
5	0.80	0.80	0.90	0.80	0.70	0.70	−0.1*	−0.6*
13	1.10	1.10	1.10	1.80	1.80	0.90	0.40	0.00

From Kelly (1993)
F, female; M, male; * $P < 0.05$; ** $P < 0.01$
[a] Dose level was decreased on test day 15 from 300 ppm to 240 ppm and then decreased again on test day 26 from 240 to 200 ppm.

Chlorfenapyr (lot no. AC 7504-59A; purity 95.4%) was fed to four groups of male and female Beagle dogs for 1 year at a dietary concentration of 0 (control), 60, 120 or 240 ppm. The control, 60 ppm and 120 ppm groups consisted of five male and five female dogs, whereas the 240 ppm group consisted of six animals of each sex. Dietary batches of chlorfenapyr at concentrations of 60 and 240 ppm were found to be stable over a period of 14 days at room temperature or frozen. Substance intake was 0, 2.1, 4.0 and 8.7 mg/kg bw per day for males and 0, 2.3, 4.5 and 10.1 mg/kg bw per day for females at 0, 60, 120 and 240 ppm, respectively.

No mortality occurred during the study. One male dog in the 240 ppm group exhibited excessive salivation during weeks 1 through 4 of the study. No other clinical signs of toxicity were noted. Body weights (Table 20) and body weight gains (Table 21) for animals in the 240 ppm group were lower than concurrent control values during the study. Body weight gains following 52 weeks of treatment for males and females in the control group were 1.6 kg and 1.5 kg, respectively, whereas weight gain for both sexes in the 240 ppm group was only 0.5 kg.

Table 20. Oral 1-year toxicity of chlorfenapyr in dog: body weight in selected weeks

Week	Body weight (% of control)							
	0 ppm		60 ppm		120 ppm		240 ppm	
	M	F	M	F	M	F	M	F
26	—	—	↑4%	↓7%	↑6%	↓6%	↓1%	↓17%
52	—	—	↑4%	↓6%	↑7%	↓8%	↑10%	↓17%

From Kelly (1994)
F, female; M, male

Table 21. Oral 1-year toxicity of chlorfenapyr in dog: body weight changes in selected weeks

Week	Body weight change (% of control)							
	0 ppm		60 ppm		120 ppm		240 ppm	
	M	F	M	F	M	F	M	F
26	—	—	↑62%	↓36%	↑54%	↓29%	0%	↓91%
52	—	—	↓19%	↓13%	↑53%	↓31%	↓69%	↓67%

From Kelly (1994)
F, female; M, male

Feed consumption and feed efficiency values of treated animals were, in general, comparable to control values. Ophthalmoscopic examinations, haematology, clinical chemistry, urine analysis and organ weight data did not reveal any indications of adverse treatment-related effects. No treatment-related macroscopic changes were observed at study termination.

For microscopic findings, the lymphoid cell population (namely, the severity rating, or the number and size of lymphoid follicles) in the stomach of several dogs in the 120 and 240 ppm groups was slightly increased compared with concurrent controls (Table 22). However, the variable severity of the lymphoid cell population may represent individual animal variation of the lymphofollicular tissue. The latter tissue has been associated with infection of one or more types of spiral-shaped bacteria (i.e. *Helicobacter felis* and/or *Gastrospirillium hominis*), believed to be part of the natural gastric flora in dogs. The lymphoid cell population in the stomach of several dogs was considered to be within normal physiological range, and the gastric lymphoid cell findings were not considered to represent a toxicologically significant effect of the compound.

Table 22. Oral 1-year toxicity of chlorfenapyr in dog: histopathological findings in the stomach

	Incidence of finding							
	0 ppm		60 ppm		120 ppm		240 ppm	
	M	F	M	F	M	F	M	F
Number of animals in the dose group	*5*	*5*	*5*	*5*	*5*	*5*	*6*	*6*
Increased lymphocytes/plasma cells, lamina propria								
- minimal	1	1	—	2	1	2	2	2
- mild	1	—	1	—	3	—	3	2
- moderate	—	—	2	—	—	—	1	—
Hypertrophy/hyperplasia of lymphoid follicles								
- minimal	3	3	—	—	—	1	—	—
- mild	2	2	3	5	2	3	1	1
- moderate	—	—	2	—	3	1	5	5
Blind reading (1)								
- minimal	4	3	1	—	—	1	—	—
- mild	—	—	1	3	1	—	1	1
- moderate	1	2	2	2	2	2	2	3
- marked	—	—	1	—	2	2	3	2
Blind reading (2)								
- minimal	3	—	1	—	1	—	—	—
- mild	2	—	3	2	—	2	1	—
- moderate	—	1	1	2	3	2	2	1
- marked	5	—	—	1	1	2	3	4

From Kelly (1994)
F, female; M, male

The NOAEL for dietary administration of chlorfenapyr to purebred Beagle dogs was 120 ppm (equal to 4.0 mg/kg bw per day for males and 4.5 mg/kg bw per day for females), based on reduced body weight and body weight gain at 240 ppm (equal to 8.7 mg/kg bw per day for males and 10.1 mg/kg bw per day for females) (Kelly, 1994).

(b) Dermal application

Chlorfenapyr technical (lot no. AC 7504-59A; purity 94.5%) was administered dermally to four groups of six male and six female New Zealand White rabbits at a dose level of 0 (control), 100, 400 or 1000 mg/kg bw per day. Animals were treated for a 6-hour period, 5 days/week, for 4 weeks.

No treatment-related mortalities or clinical signs were observed in any dose group. All animals were free of significant dermal effects and ocular changes throughout the study. Mean body weight and feed consumption values of all test animals were comparable to control values.

A very slight decrease in mean erythrocyte count was observed in females in the 1000 mg/kg bw per day dose group. Dose-related elevations in mean serum cholesterol values were evident in the 400 and 1000 mg/kg bw per day dose groups. Other treatment-related changes in clinical chemistry values were elevations in the mean serum ALT for females in the 1000 mg/kg bw per day dose group (Table 23). Urine analysis values for all treated groups were comparable to control data.

Increased absolute and relative liver weights, relative to control values, were noted for animals in the 400 and 1000 mg/kg bw per day dose groups (Table 24). Cytoplasmic vacuolation of the liver was exhibited by several animals in the 400 and 1000 mg/kg bw per day dose groups. The vacuoles were of different sizes, the affected hepatocytes did not exhibit a consistent lobular pattern and no hepatocellular necrosis was associated with this morphological change.

Table 23. Dermal 28-day toxicity of chlorfenapyr in rabbit: haematology and clinical chemistry findings

	0 mg/kg bw per day		100 mg/kg bw per day		400 mg/kg bw per day		1000 mg/kg bw per day	
	M	F	M	F	M	F	M	F
RBC (million/μl)	5.9	5.89	6.2	5.76	6.08	5.57	5.62	5.37*
Hb (g/dl)	12.6	11.9	12.8	12.1	12.5	11.6	11.7	11.4
ALT (IU/l)	43	39	54	61	65	57	46	77*
Cholesterol (mg/dl)	58	67	59	66	93*	115*	108**	131**

From Blaszcak (1993)
ALT, alanine aminotransferase; F, female; Hb, haemoglobin; IU, international units; M, male; RBC, red blood cells; * $P < 0.05$; ** $P < 0.01$

Table 24. Dermal 28-day toxicity of chlorfenapyr in rabbit: liver gross pathology and histopathology

	0 mg/kg bw per day		100 mg/kg bw per day		400 mg/kg bw per day		1000 mg/kg bw per day	
	M	F	M	F	M	F	M	F
Absolute liver weight (g)	53.59	58.26	55.45	57.78	61.82	67.77*	72.29**	78.17**
Relative liver weight (%)	2.28	2.51	2.38	2.46	2.80**	2.94**	3.05**	3.38**
Cytoplasmic vacuolation of hepatocytes	0/6	0/6	0/6	0/6	1/6	3/6	4/6	4/6

From Blaszcak (1993)
F, female; M, male; * $P < 0.05$; ** $P < 0.01$

In conclusion, the NOAEL for chlorfenapyr was 100 mg/kg bw per day, based on haematological findings and a liver weight increase together with ALT increase and cytoplasmic vacuolation in hepatocytes seen at 400 mg/kg bw per day (Blaszcak, 1993).

(c) Exposure by inhalation

Fifteen male and 15 female Wistar rats per test group were exposed (nose only) to dynamic inhalation containing dust aerosol atmospheres (Table 25) of chlorfenapyr (batch no. 2181H88HV; purity 97.8%) for 6 hours per working day, 5 days/week, for approximately 90 days (65 exposures). From the 15 animals of each sex per group, 10 were designated as main group animals and 5 as recovery group animals. The main group animals were terminated 1 day after the exposure period; the recovery group animals were terminated after a recovery period of 28 days. The doses were 0 mg/m^3 (control air) (group 0 dose / 01 recovery), 5 mg/m^3 (group 1 dose / 11 recovery), 20 mg/m^3 (group 2 dose / 21 recovery), 40 mg/m^3 (group 4 dose / 41 recovery) and 80 mg/m^3 (group 3 dose / 31 recovery).

Table 25. Inhalation 90-day toxicity of chlorfenapyr in rat: particle size data

Target concentration (mg/m^3 solids)	Measured concentration, mean ± SD (mg/m^3 solids)	Particle size distribution (MMAD), mean ± SD (µm)
Air control	n.d.	n.d.
5	5.1 ± 0.6	1.9 ± 0.2
20	20 ± 3	2.8 ± 0.1
80	79 ± 7	n.d.
40	41 ± 5	2.1 ± 0.3

From Ma-Hock et al. (2005)
MMAD, mass median aerodynamic diameter; n.d., not determined; SD, standard deviation

Inhalation of a chlorfenapyr dust aerosol led to the premature death of male animals at a concentration of 80 mg/m^3 within the first 3 exposure days (7/15 male animals: 4/10 in the main group and 3/5 in the recovery group). Because of this high lethality, surviving animals of this group were terminated prematurely. Substitute groups were exposed to 40 mg/m^3. However, one male animal was found dead after each of study day 35 and study day 80.

In addition to lethality, a slightly increased respiration rate was observed in animals exposed to 40 mg/m^3. This finding is considered to be substance related. Alopecia or injuries that were observed in individual animals were considered to be incidental.

There were no statistically significant changes in the mean body weight (Table 26) or mean body weight gain (Table 27) in animals exposed to 5 or 20 mg/m^3. Statistically significant differences were noted only in the substitute animals (groups 4 and 41). These changes in body weight and body weight gain were also considered to be incidental, as they were not concentration or time related.

Table 26. Inhalation 90-day toxicity of chlorfenapyr in rat: statistically significant changes in mean body weights

Test group	Sex	Test concentration (mg/m^3)	Study days	Change in mean body weight
4	Male	40	−4, 0 and 7	Decreased
4	Female	40	14	Increased
41	Male	40	−4 to 119	Decreased

From Ma-Hock et al. (2005)

Table 27. Inhalation 90-day toxicity of chlorfenapyr in rat: statistically significant changes in mean body weight gain

Test group	Sex	Test concentration (mg/m^3)	Study days	Change in mean body weight gain
4	Female	40	7, 14	Increased
41	Male	40	21, 28, 119	Decreased

From Ma-Hock et al. (2005)

A functional observational battery was carried out on assigned animals before exposure, during exposure (on study day 84), at the end of exposure (on study day 92) and at the end of the recovery period (study day 119). The functional observational battery started with passive observations, without disturbing the animals, followed by removal from their cage and open-field observations. Thereafter, sensorimotor tests and reflex tests were conducted. No substance-related effects were observed in home cage observations. The grip strength (forelimbs) was statistically significantly increased in males of the 40 mg/m^3 group at the end of recovery. This change was considered to be incidental and not of biological relevance. Motor activity was measured on the same day and with the same animals on which the functional observational battery was performed. The measurement was performed in the dark using the Multi-Varimex System with four infrared beams per cage. The numbers of beam interrupts were counted over 12 intervals, each lasting 5 minutes. Measurements ended exactly 60 minutes thereafter. There were some variations of motor activity (mostly increases and some decreases) seen at some intervals in both sexes. These changes were considered to be incidental, because there was no concentration–response relationship and the finding was not consistently observed among intervals.

Regarding clinical pathology findings, inhalation of the test compound at 40 mg/m^3 caused slight increases in white blood cells (Table 28) and lymphocytes at the end of the exposure period. These findings were assessed as being treatment related. At the end of the recovery period, leukocytes and lymphocytes were decreased when compared with the respective control values. The prolonged prothrombin times in the animals of both sexes exposed to 40 mg/m^3 are also considered treatment related and are indicative of slight disturbance of coagulation (Table 29).

Table 28. Inhalation 90-day toxicity of chlorfenapyr in rat: white blood cell counts

Day	White blood cells (billions/l)							
	0 mg/m^3		5 mg/m^3		20 mg/m^3		40 mg/m^3	
	M	F	M	F	M	F	M	F
93	4.62	2.76	4.69	3.52	5.21	3.22	5.92*	3.96*
120	6.11	4.88	4.56*	3.86	6.25	3.7	3.97*	2.59**

From Ma-Hock et al. (2005)
F, female; M, male; * $P < 0.05$; ** $P < 0.01$

Table 29. Inhalation 90-day toxicity of chlorfenapyr in rat: clotting analyses

Day	Clotting (HQT, s)							
	0 mg/m^3		5 mg/m^3		20 mg/m^3		40 mg/m^3	
	M	F	M	F	M	F	M	F
93	29.5	26.9	30.4	26.7	30.6	27.3	32.9**	28.7*
120	28.7	26.6	30.2	26.4	29.5	26.5	29.7	27.1

From Ma-Hock et al. (2005)
F, female; HQT, Hepato-Quick test; M, male; * $P < 0.05$; ** $P < 0.01$

Regarding pathology, the two animals dosed with 40 mg/m^3 that did not survive to termination of the study were examined by histopathology and showed autolytic changes to a different degree and marked pulmonary congestion in both cases, with one animal showing additional macroscopic lung changes (all lung lobes with diffuse discoloration, dark red). This is considered to be related to the mode of death due to asphyxiation and/or agonal respiratory distress. Considering the lethality that occurred at 80 mg/m^3, the two deaths at 40 mg/m^3 are considered to be substance related.

There were no major organ weight changes in either sex in the treated groups, and those observed were not dose related and exhibited no macroscopic or microscopic pathology. All further macroscopic or histopathological findings were regarded to be incidental in origin and not related to treatment.

No treatment-related effects were observed in animals exposed to the 5 or 20 mg/m^3 concentrations of the test compound. Thus, the no-observed-adverse-effect concentration (NOAEC) was 20 mg/m^3, based on lethality, haematological alterations and prolonged prothrombin times seen at 40 mg/m^3 (Ma-Hock et al., 2005).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Chlorfenapyr (lot no. AC 7504-59A; purity 94.5%) was fed to four groups of 65 male and 65 female CD-1 albino mice for 18 months at a dietary concentration of 0 (control), 20, 120 or 240 ppm. The compound was found to be stable in the diet after 7 and 14 days of storage in the animal room. Aliquots of diet mixes at 20 and 240 ppm were submitted to the laboratory for assessment of homogeneity; results demonstrated acceptable homogeneity. Achieved test article intakes were 0, 2.8, 16.6 and 34.5 mg/kg bw per day in males and 0, 3.7, 21.9 and 44.5 mg/kg bw per day in females, respectively. From weeks 16 to 80, the achieved intakes of test article were calculated on a weekly basis whenever feed consumption and body weights were recorded (i.e. every 2 or 4 weeks).

A statistically significant increase in mortality rate was noted for females in the 240 ppm group when compared with controls. At 18 months, survival rates for females in the control, 20 ppm, 120 ppm and 240 ppm groups were 80%, 71%, 73% and 60%, respectively (Table 30). As the severity and nature of treatment-related histopathological findings (vacuolation of the brain and dermatitis) seen in high-dose females were comparable to those seen in high-dose males, these changes were not considered to be indicative of the cause of death.

There were no neoplastic or non-neoplastic findings that could be attributed to the increase in mortality in high-dose females, and the survival rate for females in the high-dose group was not remarkably different from survival rates in historical control data from the same laboratory. A relationship between higher mortality and treatment with chlorfenapyr was considered unlikely.

Treatment with chlorfenapyr was associated with a reduced growth rate and a slightly lower feed intake in males and females receiving 240 ppm and in females receiving 120 ppm. Body weight gains over the 80-week treatment period were reduced by 30% and 14% for males and females in the 240 ppm group, respectively, and by 14% for females in the 120 ppm group. Body weight gain in selected weeks is shown in Table 31. A slightly reduced feed intake was also seen in males receiving 120 ppm.

Treatment with chlorfenapyr for 80 weeks did not produce any adverse effects on clinical signs, haematology parameters or organ weights. A slight increase in the incidence of skin ulceration and scabs was noted at necropsy for animals at the 240 ppm level. This finding was microscopically presented as dermatitis.

Table 30. Carcinogenicity study in mouse: survival rate

Week(s)	Males												Females											
	0 ppm			20 ppm			120 ppm			240 ppm			0 ppm			20 ppm			120 ppm			240 ppm		
	D	S	%	D	S	%	D	S	%	D	S	%	D	S	%	D	S	%	D	S	%	D	S	%
0	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100
0–4	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100
5–8	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100
9–12	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100
13–16	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100
17–20	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100	0	55	100
21–24	0	55	100	1	54	98	0	55	100	0	55	100	0	55	100	0	55	100	1	54	98	0	55	100
25–28	0	55	100	0	54	98	3	52	95	1	54	98	0	55	100	1	54	98	0	54	98	0	55	100
29–32	0	55	100	0	54	98	0	52	95	1	53	98	1	54	98	2	52	95	1	53	96	2	53	96
33–36	0	55	100	0	54	98	0	52	95	0	53	98	0	54	98	1	51	93	2	51	93	0	53	96
37–40	0	55	100	1	53	98	0	52	95	0	53	98	0	54	98	1	50	91	1	50	91	1	52	96
41–44	0	55	100	0	53	98	0	52	95	2	51	93	0	54	98	0	50	91	2	48	87	0	52	95
45–48	2	53	96	1	52	95	0	52	95	0	51	93	1	53	98	0	50	91	1	47	85	0	52	95
49–52	0	53	96	0	52	95	1	51	93	1	50	91	2	51	93	1	49	89	0	47	85	0	52	95
53–56	0	53	96	4	48	87	0	51	93	0	50	91	0	51	93	1	48	87	0	47	85	2	50	91
57–60	1	52	95	0	48	87	0	51	93	0	50	91	1	50	91	1	47	85	0	47	85	2	48	87
61–64	3	49	89	1	47	85	2	49	89	2	48	87	2	48	87	2	45	82	1	46	84	3	45	82
65–68	2	47	85	1	46	84	1	48	87	2	46	84	0	48	87	1	44	80	1	45	82	3	42	76
69–72	3	44	80	2	44	80	2	46	84	1	45	82	1	47	85	1	43	78	1	44	80	5	37	67
73–76	0	44	80	2	42	76	2	44	80	0	45	82	2	45	82	0	43	78	2	42	76	1	36	65
77–80	3	41	75	0	42	76	5	39	71	2	43	78	1	44	80	4	39	71	2	40	73	2	34	62
Termination	**0**	**41**	**75**	**1**	**41**	**75**	**0**	**39**	**71**	**0**	**43**	**78**	**0**	**44**	**80**	**0**	**39**	**71**	**0**	**40**	**73**	**1**	**33**	**60**

From Bernier (1994)

%, percentage of the original group size; D, number of animals dying during the period; S, number of animals surviving at the end of the period

Table 31. Carcinogenicity study in mouse: body weight gain in selected weeks

Week	Body weight gain (g)							
	0 ppm		20 ppm		120 ppm		240 ppm	
	M	F	M	F	M	F	M	F
0	3.18	1.69	2.59	1.29	2.89	1.28	2.29 B	1.53
10	0.62	0.88	0.51	0.88 D	0.55	0.22 E	0.54	0.40 B
20	0.65	0.63	0.54	0.32	0.42	0.21	0.22 E	0.21
30	0.79	1.35	0.49	0.95	0.22 C	0.56 E	0.48 b	0.61 E
42	0.99	0.68	1.07	0.13	0.36 C	0.53	0.30 C	0.21
50	0.52	−0.2	0.38	0.60 F	0.14 B	−0.12	0.24 a	0.16
62	0.42	0.8	0.64	0.42	0.56	0.66	0.39	0.52
70	−0.17	0.34	−0.01	0.26	−0.19	−0.01	−0.33	0.38
80	−0.49	−0.36	−0.25	0.32	−0.29	0.08	0.05	−0.15

From Bernier (1994)
a = $P < 0.05$ (Wilcoxon's test); b = $P < 0.01$ (Wilcoxon's test); B = $P < 0.01$ (Dunnett's); C = $P < 0.001$ (Dunnett's); D = $P < 0.05$; E = $P < 0.01$; F = $P < 0.001$ (*t*-test on adjusted means, not shown, using week 0 data as covariates)

Non-neoplastic changes associated with the test article consisted of vacuolation of the white matter of the brain. This was noted following 52 weeks of treatment in males and females receiving 240 ppm and in females receiving 120 ppm. Following 80 weeks of treatment, the incidence of vacuolation for males receiving 120 or 240 ppm was 9/39 (23%) and 33/43 (77%), respectively, and the incidence for females in these two groups was 23/40 (58%) and 33/33 (100%), respectively. Vacuolation was also noted in pre-terminal males and females receiving 240 ppm and in males receiving 120 ppm. Vacuolation was seen microscopically in spinal cord and optic nerve sections in males and females receiving 240 ppm following 80 weeks of treatment and in pre-terminal males at 240 ppm. Vacuolation of the spinal cord was also noted in pre-terminal females at 240 ppm (Table 32).

Table 32. Carcinogenicity study in mouse: selected non-neoplastic findings (n = 65 mice of each sex per group)

		0 ppm		20 ppm		120 ppm		240 ppm	
		M	F	M	F	M	F	M	F
Brain	Basophilic bodies	6	4	7	0	0	2	1	1
	Vacuolation	4	10	3	5	14	28	49	58
Liver	Focal hyperplasia	4	1	5	0	2	0	2	0
	Hypertrophy, centrilobular	0	0	1	0	4	0	4	1
	Hypertrophy, diffuse	1	0	3	0	2	0	4	0
	Inflammation, focal	19	2	16	0	10	3	7	6
Optic nerve	Vacuolation	0	0	0	0	0	1	12	14
Spinal cord lumbar	Vacuolation	0	0	0	0	2	0	11	3
Spinal cord cervical	Vacuolation	0	1	0	0	2	0	20	23
Spinal cord thoracic	Vacuolation	0	2	1	0	2	1	17	16
Skin	Dermatitis	9	3	12	1	11	6	21	9

From Bernier (1994)
F, female; M, male

The incidence of tumours was generally low, and statistical analysis of the distribution of these lesions across control and treated groups showed no evidence that their presence in any organ or tissue could be related to treatment (Table 33).

Table 33. Carcinogenicity study in mouse: selected neoplastic findings (n = 65 mice of each sex per group)

	0 ppm		20 ppm		120 ppm		240 ppm	
	M	F	M	F	M	F	M	F
Hepatocellular adenoma	8	0	10	1	4	1	2	0
Hepatocellular carcinoma	1	0	3	0	0	0	0	0
Alveolar/bronchiolar adenoma	16	7	12	7	13	8	10	9
Alveolar/bronchiolar carcinoma	2	1	1	2	3	0	4	1
Interstitial cell adenoma (testis)	1	NA	0	NA	1	NA	0	NA
Myeloid leukaemia	3	0	0	0	0	0	0	0
Adenocarcinoma (mammary gland)	NA	0	NA	2	NA	1	NA	1
Leiomyoma (uterus)	NA	5	NA	0	NA	1	NA	0
Adenocarcinoma (uterus)	NA	0	NA	0	NA	1	NA	0

From Bernier (1994)
F, female; M, male; NA, not applicable

No compound-related effects on any of the parameters evaluated were evident at the 20 ppm dietary level.

Based on the results of this study, the NOAEL for chronic toxic effects following 80 weeks of dietary administration of chlorfenapyr to mice was considered to be 20 ppm (equal to 2.8 mg/kg bw per day for males and 3.7 mg/kg bw per day for females), based on body weight effects and vacuolation of the white matter of the brain at 120 ppm (equal to 16.6 mg/kg bw per day for males and 21.9 mg/kg bw per day for females).

Treatment with chlorfenapyr at dietary levels of up to 240 ppm for 80 weeks did not produce any evidence of a carcinogenic effect. The NOAEL for oncogenic effects through 80 weeks of treatment was 240 ppm, the highest dose tested. Based on feed consumption data, this represents a daily intake of approximately 34.5 mg/kg bw per day for males and 44.5 mg/kg bw per day for females (Bernier, 1994).

Rats

Chlorfenapyr (lot no. AC 7504-59A; purity 94.5%) was fed to four groups of 65 male and 65 female Sprague-Dawley rats for 24 months at a dietary level of 0 (control), 60, 300 or 600 ppm. Achieved test article intakes were 0, 2.9, 15.0 and 30.8 mg/kg bw per day for males and 0, 3.6, 18.6 and 37.0 mg/kg bw per day for females, respectively. Formulations were stable for 14 days at room temperature and 166 days at freezer temperature.

An interim termination was conducted on 10 rats of each sex per group after 12 months of treatment. Survival was similar to or higher than that in controls and significantly increased for females at the 600 ppm level. At 24 months, survival rates were 51%, 55%, 55% and 59% for males and 36%, 35%, 44% and 58% for females in the control, 60 ppm, 300 ppm and 600 ppm groups, respectively (Table 34).

Table 34. Carcinogenicity study in rat: survival rate and historical control data

Week	Survival rate (%)									
	0 ppm		60 ppm		300 ppm		600 ppm		Historical control[a]	
	M	F	M	F	M	F	M	F	M	F
0	100	100	100	100	100	100	100	100	100	100%
52	95	98	100	100	100	97	95	100	96.88%	98.11%
104	51	36	55	35	55	44	59	58	35–67.35%	34.83–58.62%
105	59	36	55	33	54	44	55	55	—	—

From Trutter (1994)
[a] Eight studies with males and nine with females conducted between April 1984 and April 1991.

There was no evidence of a treatment-related effect from clinical observations, ophthalmoscopic findings, urine analysis results or gross pathology in any of the three test groups. Dose-related decreases in mean body weight and body weight gain were seen in the 300 and 600 ppm groups. Statistically significant decreases in body weights were noted in the 300 ppm dose group at weeks 8, 14 and 23 through 92 for males and at weeks 4, 6, 7, 10, 15 through 25, 31 through 70, 92, 100 and 105 for females. For the 600 ppm level, body weights were reduced for the majority of the treatment period. Statistically significant reductions in body weight gain were noted for both sexes at the 300 and 600 ppm levels through week 52. Body weight gains for the first 52 weeks of the study were reduced by 6.4% and 12.1% for males and by 10.5% and 21.7% for females in the 300 and 600 ppm groups, respectively. Body weight gains were also reduced through week 104 at these two levels as compared with controls; the differences were statistically significant for females. Body weight gains over the 104-week treatment period were reduced by 9.1% and 9.8% for males and by 26.4% and 29.0% for females in the 300 and 600 ppm groups, respectively (Table 35).

Table 35. Carcinogenicity study in rat: body weight gain

Week(s)	Body weight gain (g)							
	0 ppm		60 ppm		300 ppm		600 ppm	
	M	F	M	F	M	F	M	F
0	51	28	60	26	62	29	67	27
1–52	487	258	478 (↓2%)	245 (↓5%)	458* (↓6%*)	231* (↓10%*)	428* (↓12%*)	202* (↓22%*)
1–104	419	295	410 (↓2%)	298 (↑1%)	381 (↓9%)	218* (↓26%*)	378 (↓10%)	210* (↓29%*)

From Trutter (1994)
F, female; M, male; * $P < 0.05$

Total mean feed consumption for week 1 through week 52 and week 1 through week 104 and total mean feed efficiency for week 1 through week 4 were significantly reduced at the 600 ppm dietary level.

Compound-related haematology findings included statistically significant decreases in erythrocyte count, haemoglobin and haematocrit values at weeks 13, 26 and 52 for males at the 600 ppm level. Erythrocyte count and haematocrit values were significantly decreased at week 13 only for females at the 600 ppm level. Reticulocyte counts were significantly increased for males at the 600 ppm level. Statistical analysis of serum chemistry data showed significant increases in mean globulin values at weeks 13 and 26 for females in the 300 ppm group, at weeks 26 and 53 for males in the 600 ppm group and at weeks 13, 26, 53 and 78 for females in the 600 ppm group. Significant decreases in albumin to globulin ratios were also noted for males in the 600 ppm groups at weeks 26 and 78. Mean

total cholesterol values were significantly increased for females in the 300 and 600 ppm groups (Table 36).

Table 36. Carcinogenicity study in rat: haematology and serum chemistry

	0 ppm		60 ppm		300 ppm		600 ppm	
	M	F	M	F	M	F	M	F
Week 52								
RBC (millions/µl)	8.5	7.72	8.65	7.27	8.53	7.2	7.85*	7.55
Haemoglobin (g/dl)	15.5	15.1	15.7	14.8	15.8	14.1	14.1*	14.7
Haematocrit (%)	44.2	41.7	44.4	40.2	48.1	39.2	39.6*	40.6
Reticulocytes (%)	1.8	1.5	1.4	4.6	1.6	6.7	3*	1.7
Week 26								
Albumin to globulin ratio	2.08	2.92	2.02	3.04	1.90	2.29*	1.59*	2.05*
Week 78								
Cholesterol (mg/dl)	124	105	81*	120	103	145*	140	142*
Albumin to globulin ratio	1.63	2.05	1.45	1.99	1.39*	1.87	1.34*	1.55*

From Trutter (1994)
F, female; M, male; RBC, red blood cells; * $P < 0.05$

Statistical evaluation of organ weight measurements showed treatment-related increases in the liver to terminal body weight ratios in the mid-dose and high-dose males and females at 12 months and in the high-dose males and females at 24 months (Table 37). Histopathological evaluations showed compound-related alterations in the livers of the animals of the 300 and 600 ppm groups. The microscopic changes consisted of hepatocellular enlargement, which was usually of slight severity. This enlargement was observed as a centrilobular to midzonal change in animals from the mid-dose and high-dose groups terminated after 52 weeks of treatment and also occurred as a diffuse change in occasional animals from these two groups at termination (104 weeks). The hepatocellular enlargement occurred with greatest incidence in severity in the high-dose group and correlated with the increased liver to body weight ratios noted in rats of both sexes of that group (Table 38).

Statistical analysis of tumour data showed no differences that were attributable to compound administration.

Table 37. Carcinogenicity study in rat: liver weights (12 and 24 months)

	0 ppm		60 ppm		300 ppm		600ppm	
	M	F	M	F	M	F	M	F
12 months								
Liver to body weight (%)	2.342	2.630	2.544	2.703	2.672*	3.082*	2.888*	3.282*
Absolute liver weight (g)	15.96	10.16	17.09	10.53	17.28	11.01	18.02	11.43
24 months								
Liver to body weight (%)	2.547	2.814	2.670	2.820	2.937	3.093	3.301*	3.558*
Absolute liver weight (g)	16.26	12.65	16.80	12.67	16.99	11.41	18.19	12.78

From Trutter (1994)
* $P < 0.05$

Table 38. Carcinogenicity study in rat: liver histopathological findings (24 months) (n = 65 rats of each sex per group)

	0 ppm		60 ppm		300 ppm		600 ppm	
	M	F	M	F	M	F	M	F
Diffuse hepatocellular enlargement	1	3	0	1	4	5	23	13
Centrilobular hepatocellular enlargement	2	3	1	0	13	13	24	41

From Trutter (1994)
F, female; M, male

No compound-related effects on any of the parameters evaluated were evident at the 60 ppm dietary level. Thus, the NOAEL for chronic toxic effects through 24 months of treatment with chlorfenapyr was 60 ppm (equal to 2.9 mg/kg bw per day for males and 3.6 mg/kg bw per day for females), based on reduced body weight and body weight gain and increased liver weight associated with hepatocellular enlargement at 300 ppm (equal to 15.0 mg/kg bw per day for males and 18.6 mg/kg bw per day for females). Chlorfenapyr was not carcinogenic in rats when tested at doses up to 600 ppm (equal to 30.8 mg/kg bw per day for males and 37.0 mg/kg bw per day for females) (Trutter, 1994).

2.4 Genotoxicity

A summary of genotoxicity studies is given in Table 39, and details about the studies are given below. Results from an acceptable range of in vitro and in vivo genotoxicity studies showed no indication of genotoxic potential:

- Chlorfenapyr does not induce gene mutations, as it did not induce either base pair substitution or frame shift mutation in any of the tester bacterial strains.
- Chlorfenapyr was found not to induce chromosomal aberrations in Chinese hamster ovary (CHO) cells in vitro (with or without metabolic activation).
- Chlorfenapyr showed no evidence of clastogenic or polyploidy inducing activity.
- Chlorfenapyr did not induce mutations at the HGPRT locus in CHO cells.
- Chlorfenapyr did not cause chromosomal damage in vivo.
- Chlorfenapyr did not induce unscheduled deoxyribonucleic acid (DNA) synthesis (UDS), indicating no induction of DNA damage in cultured rat hepatocytes.

Chlorfenapyr technical (lot no. AC 7504-59A; purity 94.5%) was tested in standard plate incorporation assays with six bacterial tester strains. A dose range–finding assay conducted at dose levels of 100–5000 µg/plate showed excessive toxicity at all doses. Therefore, the doses tested in the mutation assay were 1, 5, 10, 25 and 50 µg/plate with and without metabolic activation by hepatic S9 preparation from Aroclor 1254–treated rats with *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and TA1538 and *Escherichia coli* WP2 *uvrA*−, along with concurrent vehicle and positive controls in two independent trials. Because of excessive toxicity at 50 µg/plate in TA1537 and TA1538, chlorfenapyr technical was retested at 0.5, 1, 5, 10, 15, 20 and 25 µg/plate in two independent trials.

Results of original and confirmation assays showed that no dose-related increases in revertant frequency or positive responses were found in the presence or absence of metabolic activation in any trial. Chlorfenapyr does not induce gene mutations, as it did not induce either base pair substitution or frame shift mutation in any of the tester bacterial strains (Mulligan, 1994a).

Table 39. Summary of genotoxicity studies of chlorfenapyr

(a) In vitro tests

Test system/method guideline	Organism/strain(s)	Levels tested	Result	Remarks	Reference
Bacterial reverse gene mutation	*Salmonella typhimurium* and *Escherichia coli*	1–50 µg/plate	No gene mutation was induced with or without metabolic activation	Bacteriotoxicity at 100 µg/plate	Mulligan (1994a)
Chromosomal aberration	CHO cell culture	1.8–225 mg/l	No chromosomal aberration was induced with or without metabolic activation	With S9 mix: 14.1 mg/l reduced the mitotic index to 39% of the control Without S9 mix: 225 mg/l reduced the mitotic index to 32% of the control	Sharma (1994a)
	Chinese hamster lung cells	1.8–225 µg/plate	No evidence of clastogenic or polyploidy inducing activity with or without metabolic activation		Adams 1994
UDS	Primary rat hepatocytes	0.05–0.3 mg/l	No UDS was induced with or without metabolic activation	Cytotoxicity at 0.5 mg/l	San (1993)
HGPRT	CHO cell culture	5–500 mg/l (250 mg/l without S9 mix)	No mutations at the HGPRT locus in CHO cells were induced with or without metabolic activation	With S9 mix: 500 mg/l caused cytotoxicity with about 50% survival Without S9 mix: 500 mg/l caused cytotoxicity with about 30% survival	Sharma (1994b)

Table 39 (continued)

(b) In vivo tests

Test system/ method guideline	Species/sex/number per group	Frequency of application	Sampling	Dose level	Results	Reference
Mouse micronucleus test	Mice/5 of each sex per group	Single oral gavage treatment	24, 48, 72 h post-dosing	Males: 7.5, 15, 30 mg/kg bw Females: 5, 10, 20 mg/kg bw	No effect on the number of micronucleated polychromatic erythrocytes in the bone marrow at any dose level at any time for males or females	Sharma (1994c)

CHO, Chinese hamster ovary; DNA, deoxyribonucleic acid; S9, 9000 × g supernatant fraction of rat liver homogenate; UDS, unscheduled DNA synthesis

Chlorfenapyr technical (lot no. AC 7504-59A; purity 94.5%) was tested in the in vitro chromosomal aberration test in Chinese hamster ovary (CHO) cells in both the presence and absence of an exogenous metabolic activation system, comprising hepatic S9 preparation from Aroclor 1254–treated rats. Based on dose range–finding results (cytotoxicity to monolayers and greater than 50% reduction in mitotic indices at higher dose levels), doses tested in the definitive assay were 6.25, 12.5, 25 and 50 µg/ml with S9 metabolic activation and 12.5, 25, 50 and 100 µg/ml without activation. Limited by toxicity in the definitive test, the doses selected and evaluated included 6.25, 12.5 and 25 µg/ml with S9 metabolic activation and 25, 50 and 100 µg/ml without activation, along with concurrent untreated vehicle and positive controls. Cells were harvested 12, 24 and 48 hours after treatment. Positive controls were included in the 24-hour harvest only. Data on the frequencies of structural chromosomal aberrations and polyploid cells were collected and analysed statistically (Table 40).

The only positive result was a significant increase in the polyploid index in CHO cells at only one concentration at the 24-hour harvest and in the presence of S9 metabolic activation. Values were in the biologically acceptable range, the linear trend in the dose–response relationship was negative and this increase was not observed at 48 hours. The activity observed does not appear to have any biological significance.

Chlorfenapyr was found to be negative in this assay and thus is not considered to be a clastogenic agent (Sharma, 1994a).

Chlorfenapyr technical (lot no. AC 7504-59A; purity 93.8%) was tested in vitro in Chinese hamster lung cells to determine whether it would cause chromosomal aberrations or polyploidy at a range of dose levels, along with concurrent controls, at three treatment periods without metabolic activation and one treatment period with metabolic activation by hepatic S9 preparation from Aroclor 1254–treated rats. Mitotic indices were calculated for cultures treated with the various concentrations of chlorfenapyr and the solvent control. High doses evaluated for chromosomal aberration analysis were selected based on 50% or greater reduction in mitotic indices in each treatment group as follows:

- 6-hour treatment with cell harvest 18 hours later at doses of 3.5, 7.0 and 14.1 µg/ml with S9 activation (set 1);
- 6-hour treatment with cell harvest 18 hours later at doses of 3.5, 7.0, 14.1 and 225 µg/ml without S9 activation (set 2);

Table 40. Chromosomal aberrations in CHO cells with chlorfenapyr technical, polyploid index, 12-, 24- and 48-hour harvest

Treatment	Flask no.	No. of cells scored	No. of polyploid cells	Polyploid index (%)	Group average (%)
12 h harvest					
With S9					
Untreated	1	100	3	3.0	
	2	100	2	2.0	2.5
Vehicle	3	100	1	1.0	
	4	100	1	1.0	1.0
25 µg/ml	5	100	1	1.0	
	6	100	1	1.0	1.0
12.5 µg/ml	7	100	0	0.0	
	10	100	1	1.0	0.5
6.25 µg/ml	11	100	0	0.0	
	12	100	0	0.0	0.0
Without S9					
Untreated	13	100	0	0.0	
	14	100	1	1.0	0.5
Vehicle	15	100	1	1.0	
	16	100	1	1.0	1.0
100 µg/ml	17	100	0	0.0	
	18	100	1	1.0	0.5
50 µg/ml	19	100	0	0.0	
	20	100	1	1.0	0.5
25 µg/ml	21	100	2	2.0	
	22	100	1	1.0	1.5
24 h harvest					
With S9					
Untreated	25	100	0	0.0	
	26	100	10	10.0	5.0
Vehicle	27	100	2	2.0	
	28	100	3	3.0	2.5
25 µg/ml	31	100	2	2.0	
	32	100	2	2.0	2.0
12.5 µg/ml	33	100	0	0.0	
	34	100	1	1.0	0.5
6.25 µg/ml	35	100	12	12.0	
	36	100	10	10.0	11.0
CP 25 µg/ml	37	100	0	0.0	
	38	100	1	1.0	0.5
Without S9					
Untreated	39	100	0	0.0	
	40	100	1	1.0	0.5

Table 40 (continued)

Treatment	Flask no.	No. of cells scored	No. of polyploid cells	Polyploid index (%)	Group average (%)
Vehicle	41	100	2	2.0	
	42	100	0	0.0	1.0
100 µg/ml	43	100	1	1.0	
	44	100	1	1.0	1.0
50 µg/ml	45	100	1	1.0	
	46	100	1	1.0	1.0
25 µg/ml	47	100	0	0.0	
	48	100	2	2.0	1.0
MMC 1 µg/ml	51	100	2	2.0	
	52	100	1	1.0	1.5
48 h harvest					
With S9					
Untreated	53	100	0	0.0	
	54	100	1	1.0	0.5
Vehicle	55	100	0	0.0	
	56	100	1	1.0	0.5
25 µg/ml	59	100	5	5.0	
	60	100	5	5.0	5.0
12.5 µg/ml	61	100	3	3.0	
	62	100	6	6.0	4.5
6.25 µg/ml	63	100	7	7.0	
	64	100	4	4.0	5.5
Without S9					
Untreated	65	100	0	0.0	
	66	100	1	1.0	0.5
Vehicle	67	100	0	0.0	
	68	100	0	0.0	0.0
100 µg/ml	69	100	2	2.0	
	70	100	1	1.0	1.5
50 µg/ml	71	100	1	1.0	
	72	100	0	0.0	0.5
25 µg/ml	73	100	2	2.0	
	74	100	2	2.0	2.0

From Sharma (1994a)
CP, cyclophosphamide; MMC, mitomycin-C; S9, 9000 × *g* supernatant fraction of rat liver homogenate

- 24-hour treatment with cell harvest at the end of the treatment period at doses of 1.8, 3.5 and 14.1 µg/ml without S9 activation (set 3);
- 48-hour treatment with harvest at the end of the treatment period at doses of 1.8, 3.5 and 7.0 µg/ml without S9 activation (set 4).

All positive control compounds caused large, statistically significant increases in the frequency of either aberrant cells or polyploid cells when compared with the solvent control.

Chlorfenapyr technical caused no statistically significant increases in the proportion of aberrant cells or polyploid cells in either the presence or absence of S9 mix at any treatment time.

It was concluded that chlorfenapyr technical showed no evidence of clastogenic or polyploidy inducing activity in this in vitro test system (Adams, 1994).

Chlorfenapyr technical (lot no. AC 7504-59A; purity 94.5%) was tested in the in vitro UDS assay in cultured rat hepatocyte cells. The concentrations tested were 0.05, 0.075, 0.1, 0.125, 0.15 and 0.3 µg/ml. Because of excessive toxicity at 0.3 µg/ml, the highest dose evaluated was 0.15 µg/ml. It should be emphasized that an intermediate concentration between 0.3 and 0.15 µg/ml should have been tested to confirm the results.

No significant increase in the incorporation of tritiated thymidine into nuclear DNA of the cultured cells was found at any dose level. Therefore, chlorfenapyr technical did not induce UDS, indicating no induction of DNA damage in cultured rat hepatocytes (San, 1993).

Chlorfenapyr technical (lot no. AC 7504-59A; purity 94.5%) was tested for inducing mutations at the HGPRT locus in CHO cells in vitro. The dose selection was based on a dose range–finding assay performed at dose levels of 10, 100, 500, 1250, 2500 and 3000 µg/ml with and without metabolic activation by hepatic S9 preparation from Aroclor 1254–treated rats. There was massive precipitation from 500 to 3000 µg/ml in the test system. In addition, the monolayers exhibited excessive toxicity at dose levels of 500 µg/ml and higher. Therefore, the mutation assay was initiated with 500 µg/ml as the high dose with S9 metabolic activation. Because of the excessive toxicity at 500 µg/ml, the doses evaluated in the mutation assay were 5, 10, 50, 100 and 250 µg/ml. The assay without metabolic activation was then conducted with test dose levels of 2.5, 5, 25, 50, 100 and 250 µg/ml.

The top dose evaluated (250 µg/ml) with and without metabolic activation demonstrated toxicity and precipitation in the test system, meeting the criteria for a maximum dose level. Statistical analysis of data from both original and confirmation assays showed that mutation frequencies at any dose were not significantly greater than concurrent control values. In addition, they were found to be within the range of background mutation frequencies observed for untreated or vehicle controls in 22 other studies performed under the same conditions. Chlorfenapyr technical therefore did not induce mutations at the HGPRT locus in CHO cells (Sharma, 1994b).

Chlorfenapyr technical (lot no. AC 7504-59A; purity 94.5%) was tested in groups of 15 male and 15 female CD-1 albino mice. Animals received a single dose by gavage at a dose level of 0 (corn oil: vehicle), 7.5, 15 or 30 mg/kg bw for males and 0, 5, 10 or 20 mg/kg bw for females. Dose levels were selected based on mortality in range-finding studies. In that experiment, there was mortality in all dose groups and no demonstration of cytotoxicity to the target organ (bone marrow cells). Therefore, in order to achieve the maximum tolerated dose, an LD_{50} was calculated from the mortality data. The top doses tested in the micronucleus assay represent 60% of the LD_{50} values for male and female mice. Furthermore, toxicokinetics studies showed that bone marrow is reached by the active substance and that dose levels were high enough to produce clinical effects.

Five animals of each sex per group were killed at 24, 48 and 72 hours following dosing for the collection of bone marrow cells. Five males dosed at 30 mg/kg bw died within 72 hours of dosing. Clinical signs of toxicity (slight diarrhoea) were observed in females dosed at 10 and 20 mg/kg bw, but no female mortality occurred at any dose level. A combination of deaths in males at the high dose and the selection of 60% of the LD_{50} values for males and females demonstrate that testing was performed at or above the maximum tolerated dose, thus satisfying the USEPA's criteria of dose selection in this assay. There was no effect on the number of micronucleated polychromatic erythrocytes in the bone marrow at any dose level at any time interval for males or females.

Therefore, chlorfenapyr did not cause chromosomal damage in vivo in this test system (Sharma, 1994c).

2.5 *Reproductive and developmental toxicity*

(a) Multigeneration studies

Chlorfenapyr (lot no. AC 7504-59A; purity 94.5%) was fed daily to four groups of Sprague-Dawley rats at a dietary concentration of 0 (control), 60, 300 or 600 ppm. Each parental generation consisted of 30 male and 30 female rats per group. Both parental generations were treated during a premating period of 10–11 weeks, and treatment continued during both a 20-day mating period and a post-mating period. Mated females continued to be treated during the ensuing gestation, lactation and post-weaning periods until termination. F_1 and F_2 litters were culled to eight pups per litter on postnatal day 4. Through both parental generations, mean test substance intake values of chlorfenapyr administered during the premating treatment periods, as derived from feed consumption data and using the nominal concentrations, approximated 5, 22 and 44 mg/kg bw per day at 60, 300 and 600 ppm, respectively. Similar test substance intake values were seen during the gestation periods; however, during the lactation periods, mean test substance intake values increased to approximately 9, 42 and 82 mg/kg bw per day for the 60, 300 and 600 ppm dietary levels, respectively. Pretest analyses of mock batches of diets at the low and high concentrations (60 and 600 ppm, respectively) confirmed that the mixing procedure developed and used for this study produced diets that were stable for at least 21 days at ambient storage.

No treatment-related mortality occurred. Parental toxicity was noted at the 300 and 600 ppm dietary levels. Reduced mean body weight gains during the mating and post-mating periods were noted for P_1 and F_1 males and females in the 600 ppm dose group. Body weight and body weight gain for the F_1 parental females in the 300 and 600 ppm dose groups were reduced (≥ 10%) during gestation, as was body weight during lactation (≥ 10%) (Table 41). There was no evidence of any parental toxicity at the 60 ppm dietary level.

Table 41. Multigeneration study in rat: parental body weight and body weight gain

	0 ppm		60 ppm		300 ppm		600 ppm	
	P_1 adults	F_1 adults	P_1 adults	F_1 adults	P_1 adults	F_1 adults	P_1 adults	F_1 adults
Body weight gain, males (% change relative to control)	—	—	↓5.9a	↑9.9b	↑2.5a	↓3.7b	↓25.3a**	↓17.8b*
Body weight, females (% change relative to control)								
- weeks 1–10^{pm}	—	—	0	↓3.8	↓3	↓9.4**	↓3.9	↓13**
- days 0, 6, 13, 20^{ge}	—	—	↑0.5	↓7.3*	↓2	↓9.6**	↓4	↓13.1**
- day 21^{la}	—	—	↓2	↓7.6**	↓2.8	↓10.7**	↓7**	↓14.2**
Body weight gain, females (% change relative to control), days 0, 6, 13, 20^{ge}	—	—	↑5.8	↓10.7	↑2	↓12.8	↓2	↓13.8*

From Schroeder (1994)

a, weeks 11–16; b, weeks 32–43; ge, gestation; la, lactation; pm, premating; * $P < 0.05$; ** $P < 0.01$ (parametric data: Anova+Dunnett's; non-parametric data: Kruskall-Wallis + Dunn's rank sum)

Reproductive performance was not affected at any dietary level of chlorfenapyr. No adverse effects at the 60, 300 or 600 ppm dietary levels were evident from reproductive indices, gestation indices or parturition data during either litter interval (Table 42).

Table 42. Multigeneration study in rat: reproductive indices

		0 ppm		60 ppm		300 ppm		600 ppm	
		M	F	M	F	M	F	M	F
Mating (% mated/total)	P_1	93.3	100	100	100	86.7	96.7	96.7	100
	F_1	82.8	90	96.7	100	90	100	93.3	100
Pregnancy (% pregnant/mated)	P_1	—	93.3	—	93.3	—	96.6	—	93.3
	F_1	—	85.2	—	86.7	—	96.7	—	96.7
Fertility (% impregnating/mated)	P_1	92.8	—	93.3	—	100	—	93.1	—
	F_1	83.3	—	89.7	—	96.3	—	96.4	—

From Schroeder (1994)
F, female; M, male

Statistically significant reductions, relative to control data, in mean pup weight (postnatal days 0 through 21) were noted for the F_1 and F_2 litters in the 300 and 600 ppm dose groups (Table 43). Pup body weights from the day of birth to weaning for F_1 and F_2 litters in the 60 ppm dose group were comparable to control data.

Table 43. Multigeneration study in rat: pup body weight

	Pup body weight (g)			
	0 ppm	60 ppm	300 ppm	600 ppm
F_1	54.1	52.9	48.9**	47.4**
F_2	51.9	50.2	48.4*	44.8**

From Schroeder (1994)
* $P < 0.05$; ** $P < 0.01$

In the F_2 high-dose group, the mean pup viability index was lower than that of the controls, and this difference was statistically significant and considered indicative of a treatment-related response. F_2 pup live birth index was unaffected by the treatment, and pup weaning index for the high-dose group over the F_2 litter interval was 100%. Thus, no adverse effect of treatment with chlorfenapyr at a dietary level up to and including 300 ppm was evident from pup survival indices during lactation for either F_1 or F_2 litters. At the 600 ppm level, the only effect of treatment was reduced pup survival over the days 0–4 interval for the F_2 litters (Table 44).

No adverse effects of treatment with chlorfenapyr were apparent from the macroscopic postmortem evaluations of the parental animals or pups, and no adverse effect of treatment at the 600 ppm dietary level was observed on microscopic evaluations of reproductive tissues, pituitary glands or macroscopic lesions.

The NOAEL for both parental and developmental toxicity was 60 ppm (equal to 5 mg/kg bw per day), based on decreased body weight at 300 ppm (equal to 22 mg/kg bw per day for parental animals) and on decreased body weight in pups at 300 ppm (equal to 22 mg/kg bw per day). The NOAEL for reproductive toxicity was 600 ppm (equal to 44 mg/kg bw per day), the highest dose tested (Schroeder, 1994).

Table 44. Multigeneration study in rat: pup index

		0 ppm	60 ppm	300 ppm	600 ppm
Pup live birth index	F_1	97.4	99.3	97.5	93.1
	F_2	98.5	97.6	98.5	96.2
Pup viability index	F_1	98.8	98.5	95.3	97
	F_2	96.2	93.5	93.5	86.5*
Pup weaning index	F_1	99.5	99.6	97.7	98.1
	F_2	97.8	98.5	98.7	100

From Schroeder (1994)
* $P < 0.05$ (parametric data: Anova+Dunnett's; non-parametric data: Kruskall-Wallis + Dunn's rank sum)

(b) Developmental toxicity

Rats

Four groups of 25 presumed pregnant Sprague-Dawley rats were used in this study. Females were presumed pregnant on the basis of observations of sperm in smears of vaginal contents and/or copulatory plugs. Chlorfenapyr technical (lot no. AC 7504-59A; purity 94.5%) was administered by gavage in an aqueous suspension with 0.5% carboxymethylcellulose at a dose level of 0 (control), 25, 75 or 225 mg/kg bw per day on day 6 through day 15 of gestation. These dose levels were chosen on the basis of results from a pilot study in which 160 mg/kg bw per day caused slightly reduced body weight gain and feed consumption in pregnant females and 270 mg/kg bw per day for 7 days caused body weight loss, reduced feed consumption and increased liver to body weight ratios in non-pregnant females.

There was no mortality or signs of toxicity in the definitive study. A dose level of 225 mg/kg bw per day resulted in significantly reduced maternal body weight gains, feed consumption and water consumption during intervals of the treatment period (Table 45). These parameters were also reduced during some intervals of the treatment period at 75 mg/kg bw per day, but the reductions were less severe than those at the higher dose level, and only those for feed consumption reached statistical significance. Maternal body weight gain, feed consumption and water consumption were not reduced at 25 mg/kg bw per day.

Ovarian, uterine and fetal observations were unaffected at all dose levels. No external, soft tissue or skeletal malformations or variations were attributable to treatment (Table 46).

Based on these results, the NOAEL for maternal toxicity was 75 mg/kg bw per day, based on decreased body weight gain at 225 mg/kg bw per day. The NOAEL for developmental toxicity was 225 mg/kg bw per day, the highest dose tested. Chlorfenapyr is neither a developmental toxicant nor a teratogenic agent in the rat (Martin, 1993).

Rabbits

Four groups of 20 artificially inseminated female New Zealand White rabbits were used in this study. Chlorfenapyr technical (lot no. AC 7504-59A; purity 94.5%) was administered by gavage in an aqueous suspension with 0.5% carboxymethylcellulose at a dose level of 0 (control), 5, 15 or 30 mg/kg bw per day on day 7 through day 19 of gestation. Rabbits were terminated on day 29 of gestation. These dose levels were selected on the basis of the results of a range-finding study in which mortality occurred at 50 mg/kg bw per day and reductions in maternal body weight, body weight gain and feed consumption occurred at 25 mg/kg bw per day.

Table 45. Developmental toxicity study in rat: maternal data

Maternal parameter	0 mg/kg bw per day	25 mg/kg bw per day	75 mg/kg bw per day	225 mg/kg bw per day
Mortality	0	0	0	0
Clinical signs	—	—	—	Localized alopecia
Feed consumption				
- g/day	21.6	20.9	20.6*	19.9**
- g/kg bw per day	69.4	68.5	67.2	65.9**
Body weight (% change relative to control)				
- day 20	—	↓2.7%	↓1.3%	↓2.4%
- day 20[a]	—	↓3%	↓1.8%	↓2.8%
Body weight gain (% change relative to control)				
- day 20	—	↓6%	↓3.5%	↓6.4%
- day 20[a]	—	↓8.4%	↓6.5%	↓10%
Gravid uterine weight (% change relative to control)	—	0%	↑4.3%	↑2.4%

From Martin (1993)
* $P < 0.05$; ** $P < 0.01$
[a] Corrected body weight = gestation body weight minus total litter weight.

Table 46. Developmental toxicity study in rat: caesarean section data

	0 mg/kg bw per day	25 mg/kg bw per day	75 mg/kg bw per day	225 mg/kg bw per day
No. of rats tested	25	25	25	25
No. of pregnant rats	22	25	24	25
Mean no. of corpora lutea	16.0	15.8	16.2	15.7
Mean no. of implantations	14.3	13.8	14.7	14.2
No. of litters with ≥ 1 live fetuses	22	25	24	25
Mean no. of resorptions, early/late	1.0/0.0	0.9/0.0	1.3/0.1	0.6/0.0
Mean no. of live fetuses	13.3	12.9	13.3	13.5
Live male fetuses (%)	52.9	50.3	52.6	50.1
Mean fetal weight (g), males/females	3.30/3.18	3.37/3.24	3.38/3.26	3.30/3.13
Litters/fetuses with any alteration	8/18	8/10	7/10	11/22

From Martin (1993)

There was no mortality or clinical signs of toxicity in the definitive study. No doe aborted or delivered prematurely during the study. Maternal body weight gain and feed consumption were reduced during the treatment period at 15 and 30 mg/kg bw per day, although the effects were not statistically significant and lacked a clear dose–response relationship (Table 47).

There was no evidence of maternal toxicity at 5 mg/kg bw per day. Ovarian, uterine and fetal observations were unaffected at all dose levels. No external, soft tissue or skeletal malformations or variations of fetuses were attributable to treatment (Table 48).

Table 47. Developmental study in rabbit: maternal body weight gain

Maternal parameter	0 mg/kg bw per day	5 mg/kg bw per day	15 mg/kg bw per day	30 mg/kg bw per day
Mortality	0	0	0	0
Body weight (% change relative to control)				
- day 29	—	0%	↓3.18%	↓1%
- day 29[a]	—	↑1.4%	↓3.3%	↑1.1%
Body weight gain (% change relative to control)				
- day 29	—	↓5.7%	↓26.5%	↓25.6%
- day 29[a]	—	↑100%	↓275%	↓22%
Gravid uterine weight (% change relative to control)	—	↓9.4%	↓3.4%	↓17.4%

From Hoberman (1993)

[a] Corrected body weight = gestation body weight minus total litter weight.

Table 48. Developmental study in rabbit: caesarean section data

	0 mg/kg bw per day	5 mg/kg bw per day	15 mg/kg bw per day	30 mg/kg bw per day
No. of rabbits tested	19	20	20	20
No. pregnant	18	19	16	17
Mean no. of corpora lutea	10.8	10.2	10.6	10.1
Mean no. of implantations	8.3	7.5	8.1	7.4
No. of litters with ≥ 1 live fetuses	17	18	16	16
Mean no. of resorptions, early/late	0.3/0.1	0.3/0.1	0.1/0.1	0.7/0.2
Mean no. of live fetuses	8.4	7.6	7.9	6.9
Live male fetuses (%)	53.7	50.2	50.2	52.2
Mean fetal weight (g), males/females	44.30/43.80	44.90/44.86	43.68/43.49	45.60/45.32
Litters/fetuses with any alteration	15/42	15/39	16/39	13/33

From Hoberman (1993)

Based on these results, the NOAEL for maternal toxicity was 5 mg/kg bw per day, based on decreased body weight gain at 15 mg/kg bw per day. The NOAEL for developmental toxicity was 30 mg/kg bw per day, the highest dose tested. Chlorfenapyr is neither a developmental toxicant nor a teratogenic agent in the rabbit (Hoberman, 1993).

2.6 Special studies

(a) Neurotoxicity

Acute neurotoxicity

Chlorfenapyr (lot no. AC 7504-59A; purity 94.5%) was administered orally, via gastric intubation, as a single dose to four groups of 10 male and 10 female Sprague-Dawley CD® rats at a dose level of 0 (control), 45, 90 or 180 mg/kg bw. These dose levels were chosen on the basis of results from a pilot study in which mortality occurred at 250 mg/kg bw for both sexes. The next

highest dose in the pilot study was 125 mg/kg bw, which produced no mortality but showed decreased activity and rapid breathing, indicating toxicity. All animals were observed for 2 weeks following treatment.

Treatment-related mortality occurred in the 180 mg/kg bw dose group. Two males and two females in this group were found dead on the day of treatment. Treatment with chlorfenapyr at dose levels up to 180 mg/kg bw did not affect body weights or feed consumption during the study.

At study day 1, there was a statistically significantly greater decrease in activity in the mid-dose group than in the control group. Because this response was not dose dependent, this decrease in the mid-dose group is considered to be incidental and not related to treatment.

Observations made during the functional observational battery evaluations on day 1 indicated that administration of chlorfenapyr at a dose level of 180 mg/kg bw resulted in changes in gait, locomotion and arousal. These changes were considered responses to the test material, as this dose was lethal in 20% of the animals. No functional observational battery findings were observed at lower (non-lethal) doses, 90 and 45 mg/kg bw (Table 49).

Table 49. Acute neurotoxicity study in rat

	0 mg/kg bw		45 mg/kg bw		90 mg/kg bw		180 mg/kg bw	
	M	F	M	F	M	F	M	F
No. of rats	10	10	10	10	10	10	10	10
Mortality	0	0	0	0	0	0	2	2
Body weight (g)	302.4	222.4	314.6	210.6	307.6	213.9	290.1	223.5
Motor activity								
Movements: mean change score[a]								
- day 1	−45.6	−60.3	−39	−50.6	−61	−60.8	−55.2	−60.4
	−52.9		−44.8		−60.9**		−57.8	
- day 8	11.9	−23.4	31.4	−19.1	4.2	−7.4	14.3	−4.1
	−5.7		6.2		−1.6		5.1	
- day 15	−1.8	−9.8	23.6	−23.7	22.4	4.3	29.4	1.2
	−5.8		−0.1		−13.4		−15.1	
Functional observation battery								
Open-field observations (day 1)								
Tremors	0	0	0	0	0	0	0	0
Locomotion impaired (slightly)	1	0	0	0	1	0	5	3
Gait incoordination (body drags)	0	0	0	0	0	0	3	0
Arousal (low)	1	0	0	1	2	0	5	3
Reflex/physiological/manipulative observations								
Touch response: no reaction	1	0	0	0	0	0	0	0
Aerial righting response: slightly uncoordinated	0	0	2	0	1	0	2	0

From Ponnock (1996)

F, female; M, male; ** $P < 0.01$

[a] Change score: algebraic difference between the post-treatment movement score and the corresponding scores at pretreatment.

Cage-side observations made following the functional observational battery evaluations also indicated that 3 of 20 animals in the 180 mg/kg bw dose group and 2 of 20 animals in the 90 mg/kg

bw dose group were lethargic on the day of treatment. These observations were not noted on the following day or at 7 or 14 days following treatment (Table 50).

Table 50. Acute neurotoxicity study in rat: cage-side observations

	0 mg/kg bw		45 mg/kg bw		90 mg/kg bw		180 mg/kg bw	
	M	F	M	F	M	F	M	F
No. of rats	10	10	10	10	10	10	10	10
Cage-side observations								
- day 1	—	—	—	—	2 lethargic	—	2 lethargic, 2 found dead	1 lethargic, 2 found dead
- day 15	1 (↓ feed consumption, ↓faecal volume)	—	1 (↓feed consumption, ↓faecal volume)[a]	1 (↓feed consumption, ↓faecal volume)[a]	—	—	—	—

From Ponnock (1996)
F, female; M, male
[a] Water valve not functioning properly.

No macroscopic or microscopic compound-related changes were observed. All areas of the brain, spinal cord and peripheral nerves from treated animals were similar to those of controls.

Based on the increased mortality noted in the 180 mg/kg bw group and clinical signs of toxicity observed in the 180 and 90 mg/kg bw groups, the NOAEL for an acute administration of chlorfenapyr was 45 mg/kg bw. Chlorfenapyr was not considered to be an acute neurotoxicant (Ponnock, 1996).

Subchronic neurotoxicity

Chlorfenapyr (lot no. AC 7504-59A; purity 94.5%) was fed to four groups of 25 male and 25 female Sprague-Dawley rats for 52 weeks at a dietary concentration of 0 (control), 60, 300 or 600 ppm. Achieved test article intakes were 0, 2.6, 13.6 and 28.2 mg/kg bw per day for males and 0, 3.4, 18.0 and 37.4 mg/kg bw per day for females, respectively. The test substance was stable in rodent feed over a 14-day period. Following 13 weeks of treatment, five rats of each sex per group were anaesthetized and then perfused in situ with formalin. After 52 weeks of treatment, 10 rats of each sex in the control and 600 ppm groups and 5 rats of each sex from the 60 and 300 ppm groups were selected for perfusion. The remaining animals were fed control diet for a 16-week recovery period. At the end of the recovery period, all surviving male rats from the control and 600 ppm groups, along with 5 and 10 randomly selected male rats from the 60 and 300 ppm groups, respectively, and 5 female rats per group were selected for perfusion. All surviving animals not selected for perfusion were terminated by carbon dioxide asphyxiation.

No treatment-related mortality occurred, and the test substance had no biologically significant effects on the parameters of the functional observational battery (Table 51), body temperature or clinical observations. Motor activity (Table 51) was not affected by treatment with chlorfenapyr. There were no necropsy observations attributable to the test substance, and alterations observed in the microscopic examination (excluding neurohistological examination, see below) of tissues from female rats were considered unrelated to chlorfenapyr.

Body weights, body weight gains and feed efficiency (Table 51) were statistically significantly reduced for animals in the 300 and 600 ppm groups either at intervals during treatment or at the end of the treatment period. These findings were more prevalent in males than in females.

During the 16-week recovery period, overall body weight gains for animals in the 300 and 600 ppm groups were higher than during the treatment period. Relative mean feed consumption values (g/kg bw per day) were reduced for animals in the 600 ppm groups during the first 2 weeks of the treatment period and tended to be increased at other intervals during the exposure period. For the entire treatment period (day 1 through day 365), relative feed consumption values were increased for both sexes in the 600 ppm group and for males in the 300 ppm group. During the recovery period, little increase in relative feed consumption values was noted for the 300 and 600 ppm groups when compared with control values.

Table 51. Subchronic neurotoxicity study in rat

	0 ppm		60 ppm		300 ppm		600 ppm	
	M	F	M	F	M	F	M	F
Number of rats	25	25	25	25	25	25	25	25
Mortality	1	3	3	1	0	0	0	1
Body weight (% change relative to control), week 52	—	—	↓0.7	↑2.5	↓8.5	↓9.7	↓13.7*	↓6.9
Body weight (% change relative to control), recovery	—	—	↓3.5	↑2.4	↓6.5	↓3.11	↓3	↑0.1
Body weight gain (% change relative to control), week 52	—	—	↓0.8	↑6	↓12	↓16.6	↓21.3**	↓11.9
Feed consumption (% change relative to control), week 52	—	—	↑2.1	↓1	↑6.2**	↑3.8	↑9.7**	↑7**
Feed consumption (% change relative to control), recovery	—	—	↓1	↓3.2	↑1	↑7.8	↑2	↑6.8
Motor activity (week 52)								
Number of movements	289.6	414.7	374.6	414.3	349.4	405.8	328.0	367.3
Time spent in movement (s)	381.3	634.4	570.3	576.1	510.1	617.6	489.8	494.8
Functional observation battery								
Number of rats	19	17	17	18	20	20	20	18
Open-field observations (week 52)								
Tremors	0	1	0	0	0	0	0	0
Vocalizations during handling	0	0	1	1	0	0	1	0
Gait normal	19	17	17	18	20	20	20	18
Arousal normal	19	17	17	18	20	20	20	18

From Foss (1994)
F, female; M, male; * $P < 0.05$; ** $P < 0.01$

Neurohistological examination of males in the 600 ppm group terminated after 13 weeks of exposure revealed swelling of the myelin sheath in the spinal roots. A more generalized myelinopathic process, consisting of vacuolar myelinopathy, vacuolation and/or myelin sheath swelling of the brain and spinal cord, was present in male rats in the 300 and 600 ppm groups terminated after 52 weeks of exposure. This process was not associated with any evidence of myelin or axon degeneration and was not evident in the male rats terminated after the recovery period (Table 52).

No adverse effects of treatment were noted at the 60 ppm dietary level. Thus, the NOAEL for a dietary exposure to chlorfenapyr was 60 ppm (equal to 2.6 mg/kg bw per day for males and 3.4 mg/kg bw per day for females), based on body weight data and neurohistopathological findings at 300 ppm (equal to 13.6 mg/kg bw per day for males and 18.0 mg/kg bw per day for females) (Foss, 1994).

Table 52. Subchronic neurotoxicity study in rat: selected neurohistological findings

	Incidence of finding							
	0 ppm		60 ppm		300 ppm		600 ppm	
	M	F	M	F	M	F	M	F
Number of rats	*10*	*10*	*5*	*5*	*5*	*5*	*10*	*10*
Vacuolar myelinopathy								
Globus pallidus	0	0	0	0	0	0	4*	0
Hippocampus	0	0	0	0	0	0	6**	0
Fimbria	0	0	0	0	1	0	7**	0
Cerebellar white matter	0	0	0	0	3*	0	5*	0
Pyramids	0	0	0	0	1	0	8**	0
Stria medullaris	0	0	0	0	1	0	6**	0
Anterior commissure	0	0	0	0	1	0	7**	0
External capsule	0	0	0	0	1	0	6**	0
Internal capsule	0	0	0	0	1	0	8**	0
Corpus callosum	0	0	0	0	1	0	7**	0
Fornix	0	0	0	0	1	0	4*	0
Cerebral peduncle	0	0	0	0	2	0	8**	0
Olfactory bulb	0	0	0	0	1	0	5*	0
Olfactory tract	0	0	0	0	0	0	5*	0
Optic nerve/chiasm	0	0	0	0	1	0	7*	0
Spinal cord, cervical	0	0	0	0	1	0	7**	0
Spinal nerve roots	1	0	0	0	4*	0	9**	0
Sciatic nerve	0	0	0	0	0	0	4*	0

From Foss (1994)
F, female; M, male; * $P < 0.05$; ** $P < 0.01$

Developmental neurotoxicity

In a developmental neurotoxicity study, chlorfenapyr was administered by gavage as an aqueous suspension in 0.5% carboxymethylcellulose to 40 female Wistar rats at 0 (control), 5, 10 or 15 mg/kg bw per day from gestation day 6 (GD 6) through lactation day 10 (LD 10). A functional observational battery was performed on 10 dams per group on GD 7, GD 14, LD 7 and LD 14. On postnatal day 4 (PND 4), litters were culled to yield four males and four females (as closely as possible). The test material was administered by gavage to pups from PND 11 through PND 21.

Offspring were allocated for detailed clinical observations (functional observational battery) and assessment of motor activity, auditory startle reflex habituation, learning and memory (water maze testing) and neuropathy at termination (PND 62). On PND 22, the whole brain was collected from 10 pups of each sex per group for histopathological examination and morphometric analysis. Pup physical development was evaluated by body weight. The age of sexual maturation (vaginal opening in females and preputial separation in males) was assessed.

No deaths or clinical signs of toxicity were reported in dams during gestation or lactation. Body weight and feed consumption were not affected by treatment. Gross necropsy of dams was unremarkable, and brain weight and morphometry were similar between treated and control groups.

Pregnancy rate, gestation length, the mean number of delivered pups per dam and percentage of liveborn and stillborn pups were not affected by treatment. Pup survival in the mid-dose group (10

mg/kg bw per day) and high-dose group (15 mg/kg bw per day) was decreased, as evidenced by an increase in the number of pups cannibalized (7 and 13, respectively, versus 1 each in the control group and low-dose group) and found dead (6 and 12, respectively, versus 4 and 3 in the control group and low-dose group, respectively). Most deaths occurred during LD 1 through LD 4 (Table 53).

Table 53. Developmental neurotoxicity study in rat: maternal and litter data

Parameter	0 mg/kg bw per day	5 mg/kg bw per day	10 mg/kg bw per day	15 mg/kg bw per day
Maternal data				
Mortality	11	19	16	15
Feed consumption (g/animal per day), gestation	18.4	18.6	19	19
Feed consumption (g/animal per day), lactation	45.1	45.5	46.1	46.1
Body weight gain (g), GDs 0–20	111.4	108.6	113.6	113.1
Body weight gain (g), LDs 0–21	35.5	30.2	36	34.7
Litter data				
Total number of litters	37	36	38	39
Pups died (%)	1.3	1	1.9	3.4
Pups cannibalized (%)	0.3	0.3	2.2*	3.7**

From Schneider et al. (2006)
* $P < 0.05$; ** $P < 0.01$

Among surviving pups, no treatment-related effects on sex ratio, clinical signs, preweaning and post-weaning body weight, functional observational battery observations or learning and memory in offspring were observed. There was a statistically significant increase in the mean latency of the acoustic startle response in male pups on PND 24 (+30%), but this was not apparent at PND 60. The mean day of achieving sexual maturation in treated animals was comparable to that in the control group. A dose-related decrease in accumulated total movement distance on PND 13 was observed in treated females; the decrease (53% of the control value) was significant in the high-dose group (15 mg/kg bw per day). It was noted that the value in this group (2273 ± 1560 cm) was comparable to the historical control range (1839–2899 cm) and that the mean concurrent control value (4321 ± 2144 cm) was above the historical control range.

Brain weight and morphometric measurements of the cerebrum and cerebellum were comparable between treated and control groups on PND 22, PND 62 and PND 111. On microscopic examination at PND 22, treatment-related minimal to moderate vacuolation of the white matter was observed in several areas of the brain, including the frontal lobe, parietal lobe, midbrain, pons, cerebellum and medulla oblongata, in up to 4 out of 10 animals of each sex in the high-dose group (15 mg/kg bw per day) compared with none in the control group. As in adults, the vacuolation was reversible upon withdrawal of treatment (from PND 22 to PND 62). The low-dose group (5 mg/kg bw per day) and mid-dose group (10 mg/kg bw per day) were examined, and no lesions were observed. No treatment-related microscopic findings were observed at the PND 62 necropsy (Table 54).

For the high-dose (15 mg/kg bw per day) female pups, although there was a slightly decreased mean size of left and right brain hippocampus (approximately 5% below control) on PND 62, this change may be due to a residual effect of the vacuolation (oedema) observed on PND 22. Furthermore, there was no decrease in mean size of either side of the brain hippocampus for males or females at the high dose (15 mg/kg bw per day) on PND 111.

Table 54. Developmental neurotoxicity study in rat: neurohistopathological findings

(a) Dams

	Histopathological finding	0 mg/kg bw per day	15 mg/kg bw per day
Number of animals in the dose group		*10*	*10*
Frontal lobe		0	0
Parietal lobe		0	0
Midbrain		0	0
Pons		0	0
Cerebellum		0	0
Medulla oblongata		0	0
Cervical cord		0	0
Gasserian ganglia		0	0
Cervical ganglia		0	0
Sciatic nerve	Axonal degeneration	0	1
Tibial nerve		0	0
Gastrocnemius		0	0
Optic nerve		0	0
Pituitary gland	Cyst	0	1

(b) Pups

	Histopathological finding	Incidence of finding (PND 22 / PND 62)							
		0 mg/kg bw per day		5 mg/kg bw per day		10 mg/kg bw per day		15 mg/kg bw per day	
		M	F	M	F	M	F	M	F
Number of animals in the dose group		*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*
Frontal lobe	Vacuolation white matter	0/0	0/0	0/—	0/—	0/—	0/—	4/0	4/0
Parietal lobe	Vacuolation white matter	0/0	0/0	0/—	0/0	0/—	0/0	2/0	4/0
Midbrain	Vacuolation white matter	0/0	0/0	0/—	0/—	0/—	0/—	0/0	1/0
Pons	Vacuolation white matter	0/0	0/0	0/—	0/—	0/—	0/—	1/0	2/0
Cerebellum	Vacuolation white matter	0/0	0/0	0/—	0/—	0/—	0/—	1/0	3/0
Medulla oblongata	Vacuolation white matter	0/0	0/0	0/—	0/—	0/—	0/—	0/0	1/0
Cervical cord		0/0	0/0	—	—	—	—	0/0	0/0
Gasserian ganglia		0/0	0/0	—	—	—	—	0/0	0/0
Cervical ganglia		0/0	0/0	—	—	—	—	0/0	0/0
Sciatic nerve	Axonal degeneration	0/0	0/0	—	—	—	—	0/1	0/1
Proximal tibial nerve	Axonal degeneration	0/1	0/0	—	—	—	—	0/0	0/0
Distal tibial nerve	Axonal degeneration	0/2	0/0	—	—	—	—	0/0	0/0
Gastrocnemius	Myofibrillar degeneration	0/1	0/0	—	—	—	—	0/0	0/0

Histopathological finding	Incidence of finding (PND 22 / PND 62)							
	0 mg/kg bw per day		5 mg/kg bw per day		10 mg/kg bw per day		15 mg/kg bw per day	
	M	F	M	F	M	F	M	F
Optic nerve	0/0	0/0	—	—	—	—	0/0	0/0
Pituitary gland	0/0	0/0	—	—	—	—	0/0	0/0

From Schneider et al. (2006)
—, not assessed; F, female; M, male; PND, postnatal day

Based on the results and findings noted above, the critical effect of multifocal vacuolation (minimal to moderate severity) of the white matter of the brain observed in rat offspring resulted in an offspring NOAEL of 10 mg/kg bw per day and an offspring lowest-observed-adverse-effect level (LOAEL) of 15 mg/kg bw per day. The maternal NOAEL was 15 mg/kg bw per day, the highest dose tested (Schneider et al., 2006).

(b) Pharmacological studies

Studies on the pharmacological action of chlorfenapyr were performed in mice, rats and rabbits to evaluate effects on the central nervous system, respiratory and cardiovascular systems, autonomic nervous system, gastrointestinal system, skeletal muscle and blood coagulation.

The effects of chlorfenapyr on general behaviour were evaluated following oral administration to three male animals per dose group as follows: vehicle control; 0.3, 1, 3, 10, 30 and 100 mg/kg bw in mice; 3, 10, 30, 100, 300 and 1000 mg/kg bw in rats.

At a dose of 3 mg/kg bw in mice, one of three animals was affected for any behavioural element: depression of grooming behaviour and reactivity, decrease of spontaneous motor activity and abnormal gait. At a dose of 10 mg/kg bw, gait was normal, whereas the frequency of prone position and slight diarrhoea increased. All these changes disappeared within 6 hours. Doses of 30 and 100 mg/kg bw induced the same behaviour changes mentioned above (except diarrhoea) to a moderate degree, with dilation of the pupil and salivation as well. At a dose of 30 mg/kg bw, one of the three mice died, and at 100 mg/kg bw, all three mice died after clonic convulsions. Oral administration of chlorfenapyr to three males per dose group (vehicle control, 1, 3, 10 mg/kg bw) had no effect on hexobarbital sleeping time in mice.

In rats, a dose of 30 mg/kg bw induced a slight decrease of spontaneous motor activity and frequency of prone position; a dose of 100 mg/kg bw and above also induced depression of grooming behaviour and reactivity, abnormal limb position and gait, and salivation and an increase in body temperature. All of the rats died after clonic convulsions within 6 hours after administration of 100 mg/kg bw and within 4 hours at 300 and 1000 mg/kg bw. Oral administration of chlorfenapyr to three male rats per dose group (vehicle control, 3, 10, 30 mg/kg bw) had no effect on rectal temperature in the low- and mid-dose groups. At 30 mg/kg bw, rectal temperature was elevated from 1 to 5 hours, with a peak increase of 1.5 °C at 3 hours. Rectal temperature had returned to normal by 7 hours.

In rabbits, oral administration of chlorfenapyr to three males per dose group (doses: 3, 10 and 30 mg/kg bw) showed no effects on the spontaneous electroencephalogram. In anaesthetized rabbits, chlorfenapyr administered intraduodenally to three males per dose group (doses: 3, 10 and 30 mg/kg bw) had no effect on respiration, blood pressure, heart rate or the electrocardiogram over the 4-hour observation period.

Effects on the autonomic nervous system were evaluated in six male rats per dose group by oral administration of chlorfenapyr at doses of 3, 10 and 30 mg/kg bw. No effect on pupil size was observed.

Chlorfenapyr administered orally (doses: 1, 3 and 10 mg/kg bw) to eight fasted male mice per dose group had no effect on the intestinal transport of a charcoal meal.

Effects on the skeletal muscle were evaluated in eight male mice per dose group by oral administration of chlorfenapyr (doses: 1, 3 and 10 mg/kg bw) using a horizontal wire traction test. No changes in muscle contractility were observed.

A study to evaluate effects on blood coagulation was performed in six male rats per dose group. Three hours after oral administration of chlorfenapyr (doses: 3, 10 and 30 mg/kg bw), blood samples were collected to measure the prothrombin time (PT) and the activated partial thromboplastin time (APTT). A dose of 30 mg/kg bw significantly prolonged the PT, but this change was not considered to be of consequence, because the extent to which PT was prolonged, less than 2 seconds, is considered to be biologically insignificant.

In conclusion, chlorfenapyr had no biologically important effects on the respiratory or cardiovascular system, autonomic nervous system, skeletal muscle system, gastrointestinal system or blood coagulation. Some pharmacological effects were observed on the central nervous system, such as changes in general behaviour and an increase in body temperature. The minimum doses to produce changes in general behaviour were 3 mg/kg bw in mice (Table 55) and 30 mg/kg bw in rats (Table 56). Effects in mice at 3 mg/kg bw were marginal and related to one animal only. Convulsions due to stimulation of the central nervous system were thought to be the cause of death observed in rats and mice after acute intoxication. No changes in the electroencephalogram were observed at non-lethal doses in rabbits. No-effect doses (NOAELs) were 3 mg/kg bw in mice, 10 mg/kg bw in rats and 30 mg/kg bw in rabbits (Horii, 1994).

(c) Toxicity of metabolites

Acute oral studies on metabolites

The acute oral toxicity of metabolites of chlorfenapyr is summarized in Table 57.

AC 312,094 (residue present in fish). In an acute oral toxicity study, five rats (Charles River Crl:CD(SD)BR) of each sex per dose received AC 312,094 (pyrrole-3-carbonitrile, 2-(*p*-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)) (lot no. AC 8698-67A; purity 96.3%) dispersed in 0.5% w/v carboxymethylcellulose and sterile distilled water by gavage at a dose level of 5000 mg/kg bw. The protocol was in compliance with USEPA test guideline 81-1.

Deaths occurred in a male on day 4 (1/5 males) and in a female on day 6 (1/5 females). Clinical signs were limited to decreased activity in males only; the animals returned to normal by 24 hours post-dosing. Body weight in surviving animals was unaffected by administration of the test metabolite. There were no gross pathological changes in decedents or surviving animals. The LD_{50} was greater than 5000 mg/kg bw (Bradley, 1994b).

AC 303,268 (residue present in rat, hen and goat). In an acute oral toxicity study, five rats (Charles River Crl:CD(SR)BR) of each sex per dose received AC 303,268 (pyrrole-3-carbonitrile, 4-bromo-2-(*p*-chlorophenyl)-5-(trifluoromethyl)) (lot no. AC 8697-44B; purity 100.3%) dispersed in 0.5% w/v carboxymethylcellulose and sterile distilled water by gavage at initial dose levels of 250 and 125 mg/kg bw. Because of excessive mortality, dose levels of 62.5, 31.25, 15.6 and 7.8 mg/kg bw were tested. An additional dose level of 23.4 mg/kg bw was tested in order to calculate an LD_{50}. The protocol was in compliance with USEPA test guideline 81-1. Deaths occurred in both sexes by 8 hours post-dosing at 31.25 mg/kg bw and above.

Prostration with hind legs extended was observed in one surviving animal at doses of 31.25, 62.5 and 125 mg/kg bw, but returned to normal within 24 hours post-dosing. Other clinical signs were limited to testes protruding, writhing and tonus at necropsy in decedents. There were no relevant findings on body weight. Gross pathological changes in decedents consisted of dark or mottled livers and pronounced muscle striations. The LD_{50} was 27 mg/kg bw in males and 29 mg/kg bw in females (Bradley, 1994c).

Table 55. Effect of chlorfenapyr on general behaviour in male mice[a]

	Incidence of finding[b]																											
	3 mg/kg bw							10 mg/kg bw							30 mg/kg bw							100 mg/kg bw						
	0.5 h	1 h	2 h	4 h	6 h	8 h	10 h	0.5 h	1 h	2 h	4 h	6 h	8 h	10 h	0.5 h	1 h	2 h	4 h	6 h	8 h	10 h	0.5 h	1 h	2 h	4 h	6 h	8 h	10 h
Depression of grooming	—	1/3	—	—	—	—	—	1/3	3/3	3/3	—	—	—	—	3/3	3/3	3/3	3/3	1/2	1/2	—	3/3	1/1	—	—	—	—	—
Depression of reactivity	—	—	1/3	1/3	—	—	—	—	—	3/3	3/3	—	—	—	—	—	3/3	3/3	1/2	1/2	—	1/3	1/1	—	—	—	—	—
Decrease of SMA	—	1/3	—	—	—	—	—	1/3	3/3	3/3	3/3	—	—	—	3/3	3/3	3/3	3/3	1/2	1/2	1/2	3/3	1/1	—	—	—	—	—
Abnormal body position	—	—	—	—	—	—	—	—	—	1/3	1/3	—	—	—	3/3	3/3	3/3	2/3	1/2	1/2	1/2	3/3	1/1	—	—	—	—	—
Abnormal gait	—	—	—	1/3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1/3	—	—	—	—	—	—
Increase of pupil size	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2/3	1/1	—	—	—	—	—
Salivation	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2/3	1/1	—	—	—	—	—
Diarrhoea	—	—	—	—	—	—	—	—	—	1/3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Death	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1/3	—	—	—	2/3	3/3	—	—	—	—

From Horii (1994)
SMA, spontaneous motor activity
[a] No effects observed at 0.3 and 1 mg/kg bw.
[b] Number of animals showing abnormal clinical sign/number of animals used.

Table 56. Effect of chlorfenapyr on general behaviour in male rats[a]

Clinical signs	Incidence of finding[b]																											
	30 mg/kg bw							100 mg/kg bw							300 mg/kg bw							1000 mg/kg bw						
	0.5 h	1 h	2 h	4 h	6 h	8 h	10 h	0.5 h	1 h	2 h	4 h	6 h	8 h	10 h	0.5 h	1 h	2 h	4 h	6 h	8 h	10 h	0.5 h	1 h	2 h	4 h	6 h	8 h	10 h
Depression of grooming	—	—	—	—	—	—	—	—	—	3/3	—	—	—	—	—	2/3	3/3	—	—	—	—	2/3	3/3	3/3	—	—	—	—
Depression of reactivity	—	—	—	—	—	—	—	—	—	3/3	1/1	—	—	—	—	2/3	3/3	—	—	—	—	2/3	2/3	3/3	—	—	—	—
Decrease of SMA	—	—	2/3	3/3	—	—	—	—	—	3/3	1/1	—	—	—	1/3	2/3	3/3	—	—	—	—	2/3	3/3	3/3	—	—	—	—
Convulsions	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1/3	—	—	—	—
Abnormal body position	—	—	2/3	3/3	—	—	—	—	—	3/3	1/1	—	—	—	—	2/3	3/3	—	—	—	—	2/3	3/3	3/3	—	—	—	—
Abnormal limb position	—	—	—	—	—	—	—	—	—	—	1/1	—	—	—	—	—	3/3	—	—	—	—	—	—	3/3	—	—	—	—
Abnormal gait	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2/3	—	—	—	—	—	—	3/3	—	—	—	—
Decrease of limb tone	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2/3	—	—	—	—	—
Salivation	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1/3	—	—	—	—	—	—	2/3	—	—	—	—
Increase of body temperature	—	—	—	—	—	—	—	—	—	2/3	1/1	—	—	—	—	2/3	3/3	—	—	—	—	—	3/3	3/3	—	—	—	—
Diarrhoea	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2/3	1/3	1/3	—	—	—	—
Death	—	—	—	—	—	—	—	—	—	—	2/3	3/3	—	—	—	—	—	3/3	—	—	—	—	—	—	3/3	—	—	—

From Horii (1994)
SMA, spontaneous motor activity
[a] No effects observed at 3 or 10 mg/kg bw.
[b] Number of animals showing abnormal clinical sign/number of animals used.

Table 57. Summary of oral acute toxicity of chlorfenapyr metabolites in the rat

Metabolite	LD_{50} (mg/kg bw)		Purity (%)	Lot no.	Reference
	Males	Females			
AC312,094	> 5000	> 5000	96.3	AC8698-67A	Bradley (1994b)
AC303,268	27	29	100.3	AC8697-44B	Bradley (1994c)
AC322,250	> 5000	2500	89.0	AC9014-97A	Bradley (1994d)
AC325,195	776	1367	97.0	AC9014-93B	Bradley (1994e)

AC 322,250 (residue present in goat). In an acute oral toxicity study, five rats (Charles River CRL:CD®BR) of each sex per dose received AC 322,250 (pyrrole-2-carboxylic acid, 3-bromo-5-(*p*-chlorophenyl)-4-cyano-) (lot no. AC 9014-97A; purity 89.0%) dispersed in 0.5% w/v carboxymethylcellulose and sterile distilled water by gavage at a dose level of 5000 mg/kg bw. Because of high mortality of female rats, two additional dose groups of 2500 and 1250 mg/kg bw were added, with five female rats per group. The protocol was in compliance with USEPA test guideline 81-1. Deaths occurred at the 5000 and 2500 mg/kg bw dose levels by 5 days post-dosing. All females at the 1250 mg/kg bw dose level survived the 14 days post-dosing (5000 mg/kg bw: 1/5 males, 4/5 females; 2500 mg/kg bw: 4/5 males; 1250 mg/kg bw: 0/5 females). Clinical signs were limited to animals at the 5000 mg/kg bw dose level and consisted of decreased activity, diarrhoea, diuresis and ptosis. Surviving animals returned to normal between 24 and 48 hours post-dosing. There were no gross pathological changes in surviving animals that could be attributed to test material administration. Necropsy findings included a dark spleen, a test material–filled stomach and a gas-filled stomach in decedents. There were no relevant findings on body weight. The LD_{50} was greater than 5000 mg/kg bw in males and 2500 mg/kg bw in females (Bradley, 1994d).

AC 325,195 (residue present in rat, hen and goat). In an acute oral toxicity study, five rats (Charles River CRL:CD®BR) of each sex per dose received AC 325,195 (2-pyrrolidine-3-carbonitrile, 2-(*p*-chlorophenyl)-5-hydroxy-4-oxo-5-(trifluoromethyl)-) (lot no. AC 9014-93B; purity 97.0%) in 0.5% w/v carboxymethylcellulose and sterile distilled water by gavage at dose levels of 312.5 mg/kg bw (males only), 625, 1250, 2500 and 5000 (both sexes). The protocol was in compliance with USEPA test guideline 81-1. Deaths generally occurred in both sexes from 2 to 24 hours post-dosing. Females at the 625 mg/kg bw and males at the 312.5 mg/kg bw dose level survived the 14-day study period (312.5 mg/kg bw: 0/5 males; 625 mg/kg bw: 2/5 males, 0/5 females; 1250 mg/kg bw: 4/5 males, 3/5 females; 2500 mg/kg bw: 5/5 males, 4/5 females; 5000 mg/kg bw: 5/5 males, 5/5 females). Major clinical signs included salivation, decreased activity, hyperthermia, chromodacryorrhoea, dyspnoea, ptosis, brown material around the nose, red material in urine, dehydration and prostration in decedents. Clinical signs in surviving animals were limited to ptosis, diuresis and brown material around the nose. There were no clinical signs observed in females at the 625 mg/kg bw dose level or in males at the 312.5 mg/kg bw dose level. Gross pathological changes observed in decedents consisted of white foci on the liver and spleen and discoloration of the spleen. There were no relevant findings on body weight. Treatment-related lesions in the liver and spleen were observed at gross necropsy for three animals that were found dead at the 1250 and 2500 mg/kg bw dose levels. Microscopic evaluation of tissues of these animals showed areas of hepatocellular necrosis and/or infarction and diffuse haemorrhage. There were no gross pathological changes in surviving animals that could be attributed to ingestion of the test material. The LD_{50} was 776 mg/kg bw in males and 1367 mg/kg bw in females (Bradley, 1994e).

Genotoxicity studies on metabolites

Based on a dose range–finding test showing a limit of solubility (evidence of precipitate at the highest dose) in the test system, AC 312,094 was evaluated for its ability to produce mutations in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 and in *E. coli* strain WP2 *uvrA*$^-$ at concentrations of 25, 50, 100, 250, 500 and 1000 µg/plate with metabolic activation by hepatic S9 preparation from Aroclor 1254–treated rats and 5, 10, 25, 50, 100 and 250 µg/plate without metabolic

activation, along with concurrent negative and positive controls. Results obtained in these assays indicated that the test material was not mutagenic, in that it did not induce either base pair substitution or frame shift mutations in any of the tester strains of bacteria with or without metabolic activation (Mulligan, 1994b).

Based on dose range–finding studies, AC 303,268 was tested in *E. coli* tester strain WP2 *uvrA*$^{-}$ at concentrations of 10, 25, 50, 100 and 250 µg/plate (limited by solubility; evidence of precipitate in the test system at the highest dose) and 0.05, 0.10, 0.25, 0.5, 1.0 and 5.0 µg/plate (limited by toxicity) in the remaining tester strains (*S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538) with and without metabolic activation by hepatic S9 preparation from Aroclor 1254–treated rats, along with concurrent negative and positive controls. Results obtained in these assays indicated that the test material was not mutagenic, in that it did not induce either base pair substitution or frame shift mutations in any of the tester strains of bacteria with or without metabolic activation (Mulligan, 1994c).

Based on a dose range–finding test, AC 322,250 was tested at concentrations of 250, 500, 1000, 2500 and 5000 µg/plate in *E. coli* strain WP2 *uvrA*$^{-}$ with and without metabolic activation by hepatic S9 preparation from Aroclor 1254–treated rats. The remaining strains (*S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538) were tested at concentrations of 100, 250, 500, 1000 and 2500 µg/plate with S9 metabolic activation and at 50, 100, 250, 500 and 1000 µg/plate without S9 metabolic activation, along with concurrent negative and positive controls. The highest doses in the test system with the *Salmonella* strains were limited by toxicity. Results obtained in these assays indicated that AC 322,250 was not mutagenic, as it did not induce either base pair substitution or frame shift mutations in any of the tester strains of bacteria with or without metabolic activation (Mulligan, 1994d).

Dermal absorption study

The absorption, distribution and excretion of radioactivity were studied in male rats following a single dermal administration of ^{14}C-labelled BAS 306 I (chlorfenapyr) in the formulation concentrate BAS 306 02 I or a 1/100 (w/w) aqueous dilution of the formulation concentrate, representing an application dilution. The nominal dose levels were 2.4 or 0.0217 mg/cm^2 (corresponding to approximately 24 or 0.217 mg/animal and approximately 90 or 0.9 mg/kg bw). Groups of four animals each were exposed according to the following regimen:

exposure duration (hours):	8	8	8
skin wash after (hours):	8	8 + 24	8 + 120
sacrifice time (hours):	8	24	120

The following specimens/tissues were collected for total radioactivity: urine, faeces, blood cells, plasma, carcass, skin at the application site and skin surrounding application site. For balance estimates, the cage wash and skin washes, as well as the protective cover, were also checked for radioactivity.

Mean recoveries of radioactivity from all dose groups were in the range of 91.67–104.94% of the total radioactivity administered. The largest proportion of radioactivity was recovered from the skin wash. At the high dose (the formulation concentrate), a relative absorption of about 0.32% was observed after an 8-hour exposure period. At sacrifice after 24 and 120 hours, the relative absorptions were 0.66% and 1.45% of the dose, respectively. At the low dose (representing typical application dilution), a relative absorption of about 3.34% was observed after an 8-hour exposure period. At sacrifice after 24 and 120 hours, the relative absorptions were 7.80% and 14.26%, respectively.

The dermal penetration rate of the diluted product in rats was approximately 15% of the applied dose at 120 hours after an 8-hour exposure period (Fabian & Leibold, 2005).

3. Observations in humans

Chlorfenapyr technical has been produced since 1994 at one location in the USA. During this period, there have been no unusual or abnormal health effects observed among the personnel working in the plant. BASF Corporation policy mandates periodic medical monitoring of all production personnel, typically either annually or biannually. There are no medical tests specific to the handling of, or exposure to, chlorfenapyr.

Comments

Biochemical aspects

In two metabolism studies, one of which was not certified to be compliant with GLP, chlorfenapyr labelled with ^{14}C in either the pyrrole or the phenyl ring was administered by oral gavage to intact and bile duct–cannulated rats. The radiolabel was relatively slowly absorbed, the extent varying from 80% at 2 mg/kg bw to 65% at 20 mg/kg bw. The maximum concentration of radiolabel in plasma was achieved after about 8–12 hours, was dose proportional (at 2–20 mg/kg bw) and did not differ between males and females. Absorbed radiolabel was slowly distributed throughout the body, with concentrations in fat, liver and adrenals being greater than those in plasma. In general, tissue radiolabel concentration increased with dose. Blood and tissue concentrations of radiolabel were 2- to 3-fold higher in female rats than in male rats. Excretion was relatively rapid, mainly via the faeces, ranging from 80% to 106% of the administered dose in 7 days. There was little or no potential for accumulation, with 70% of the dose excreted in 24 hours and approximately 90% within 48 hours. The elimination half-life for plasma radiolabel was approximately 56 hours. Most of the chlorfenapyr in faeces was present as the unchanged compound, comprising material that was not absorbed together with material excreted via the bile, which was the main route of elimination. Faeces also contained minor amounts of *N*-dealkylated, debrominated and hydroxylated oxidation products of chlorfenapyr. Excretion via the urine was minor, representing only 5–11% of the administered dose over 7 days. There was no elimination of chlorfenapyr-related radioactivity via respiration.

The major routes of metabolism are *N*-dealkylation, dehalogenation, hydroxylation and conjugation, but not with sulfate or glucuronide. There is no cleavage of the bond between the pyrrole and phenyl rings of chlorfenapyr during its biotransformation.

Toxicological data

Chlorfenapyr technical is moderately toxic via the oral route, with LD_{50}s of 441 mg/kg bw in rats and 45 mg/kg bw in mice, and via the inhalation route, with an LC_{50} of 0.83 mg/l in rats. Chlorfenapyr was of low toxicity after dermal exposure in rabbits (LD_{50} > 2000 mg/kg bw). It is not irritating to the skin or eye of rabbits and is not a dermal sensitizer in the guinea-pig maximization test.

Following repeated administration of chlorfenapyr to mice, rats and dogs, decreased feed consumption and body weight gains were observed in all three species. Increased liver weights, associated with hepatocellular hypertrophy, and vacuolation in the brain and spinal cord were also noted in rats and mice.

In a 28-day study in mice, the NOAEL was 160 ppm (equal to 30.1 mg/kg bw per day), based on decreased body weight gain, mortality and increased relative liver weight at 240 ppm (equal to 43.6 mg/kg bw per day). In a 90-day study in mice, the NOAEL was 80 ppm (equal to 14.8 mg/kg bw per day), based on increased relative spleen weight and myelopathy in brain and spinal cord in males at 160 ppm (equal to 27.6 mg/kg bw per day).

In a 28-day study in rats, the NOAEL was 600 ppm (equal to 68.3 mg/kg bw per day), based on increases in relative liver weights and ALT activity at 900 ppm (equal to 106.3 mg/kg bw per day). In a 90-day study in rats, the NOAEL was 300 ppm (equal to 22 mg/kg bw per day), based on increases in relative liver weight, alkaline phosphatase activity and blood urea nitrogen and, in females, changes in red cell parameters (haemoglobin) at 600 ppm (equal to 44.9 mg/kg bw per day).

Vacuolation of the brain and spinal cord was seen at higher doses (900 ppm, equal to 69.5 mg/kg bw per day, and above).

In a 90-day dietary study in dogs, the NOAEL was 120 ppm (equal to 3.9 mg/kg bw per day), based on decreased body weight gain at 200 ppm (equal to 7.1 mg/kg bw per day). In a 1-year dietary study in dogs, the NOAEL was 120 ppm (equal to 4.0 mg/kg bw per day), based on reduced body weight and body weight gain at 240 ppm (equal to 8.7 mg/kg bw per day). The overall NOAEL for these two studies in the dog was 4 mg/kg bw per day.

Long-term studies of toxicity and carcinogenicity were performed in mice and rats, with similar NOAELs in the two species. In an 18-month dietary study in mice, the NOAEL for non-neoplastic effects was 20 ppm (equal to 2.8 mg/kg bw per day), based on decreases in body weight gain and vacuolation of the white matter of the brain at 120 ppm (equal to 16.6 mg/kg bw per day). No evidence of carcinogenicity was found.

In a 24-month dietary study in rats, the NOAEL for non-neoplastic effects was 60 ppm (equal to 2.9 mg/kg bw per day), based on reduced body weight and body weight gain and increased liver weight associated with hepatocellular hypertrophy at 300 ppm (equal to 15 mg/kg bw per day). No evidence of carcinogenicity was found.

The Meeting concluded that chlorfenapyr was not carcinogenic in rats and mice.

The potential genotoxicity of chlorfenapyr was tested in an adequate range of in vitro and in vivo studies. Chlorfenapyr showed no evidence of genotoxicity.

The Meeting concluded that chlorfenapyr was unlikely to be genotoxic in vivo.

On the basis of the lack of genotoxicity and the absence of carcinogenicity in the rat and the mouse, the Meeting concluded that chlorfenapyr is unlikely to be carcinogenic in humans.

In a two-generation reproductive toxicity study in rats, the NOAEL for effects on fertility was 600 ppm (equal to 44 mg/kg bw per day), the highest dose tested. The NOAEL for parental toxicity was 60 ppm (equal to 5 mg/kg bw per day), based on decreased body weight and body weight gain at 300 ppm (equal to 22 mg/kg bw per day). The NOAEL for offspring toxicity was 60 ppm (equal to 5 mg/kg bw per day), based on decreased body weight of pups at 300 ppm (equal to 22 mg/kg bw per day).

In a developmental toxicity study in rats, the NOAEL for maternal toxicity was 75 mg/kg bw per day, based on decreased body weight at 225 mg/kg bw per day. The NOAEL for developmental toxicity was 225 mg/kg bw per day, the highest dose tested.

In a developmental toxicity study in rabbits, the NOAEL for maternal toxicity was 5 mg/kg bw per day, based on decreased body weight gain at 15 mg/kg bw per day. The NOAEL for developmental toxicity was 30 mg/kg bw per day, the highest dose tested.

The Meeting concluded that chlorfenapyr was not teratogenic.

In an acute neurotoxicity study in rats, the NOAEL for systemic toxicity was 45 mg/kg bw, based on clinical signs of toxicity (2 lethargic animals out of 20) at 90 mg/kg bw and above. Lethality (20%) was observed at 180 mg/kg bw. There was no evidence for neuropathological effects or neurotoxicity up to the highest dose tested (180 mg/kg bw).

In a 1-year neurotoxicity study in rats, the NOAEL for neurotoxicity was 60 ppm (equal to 2.6 mg/kg bw per day), based on vacuolar myelinopathy, vacuolation and/or myelin sheath swelling of the brain and spinal cord in males at 300 ppm (equal to 13.6 mg/kg bw per day). There was no change in motor activity or other behavioural activity. The effects observed were reversible within 16 weeks.

In a developmental neurotoxicity study in rats, the NOAEL for maternal toxicity was 15 mg/kg bw per day, the highest dose tested. The NOAEL for developmental neurotoxicity was 10 mg/kg bw per day, based on an increased incidence of multifocal vacuolation (minimal to moderate severity) of the white matter of the brain on postnatal day 22 at 15 mg/kg bw per day. This effect

appears to be reversible (i.e. 38 days after end of treatment), as no adverse effects on either behaviour or neuropathology were evident in rats on PND 60.

Single-dose studies on the pharmacological action of chlorfenapyr were performed in mice, rats and rabbits to evaluate effects on the central nervous system, autonomic nervous system, respiratory and cardiovascular systems, gastrointestinal system, skeletal muscle and blood coagulation. The only relevant pharmacological effects were observed on the central nervous system, such as changes in general behaviour and an increase in body temperature. Convulsions due to stimulation of the central nervous system were thought to be the cause of death observed in rats and mice after acute intoxication. No changes in the electroencephalogram were observed at non-lethal doses in rabbits. NOAELs were 3 mg/kg bw in mice, 10 mg/kg bw in rats and 30 mg/kg bw (the highest dose tested) in rabbits, based on depression of grooming behaviour and reactivity, a decrease in spontaneous motor activity and prone position in mice and rats.

The acute oral toxicity of four chlorfenapyr animal metabolites (AC 312,094, AC 303,268, AC 322,250 and AC 325,195) was tested in Sprague-Dawley rats, and its genotoxicity was tested in microbial test systems.

AC 312,094 was of low acute oral toxicity in rats (LD_{50} > 5000 mg/kg bw) and showed no mutagenic potential in microbial test systems.

AC 303,268 was of high acute oral toxicity in rats (LD_{50} = 27 mg/kg bw). This metabolite showed no mutagenic potential in microbial test systems. It is present at significant levels in livestock.

AC 322,250 was of slight acute oral toxicity in rats (LD_{50} = 2500 mg/kg bw) and showed no mutagenic potential in microbial test systems.

AC 325,195 was of moderate acute oral toxicity in rats (LD_{50} = 776 mg/kg bw) and showed no mutagenic potential in microbial test systems.

There were no reports of adverse health effects of chlorfenapyr in manufacturing plant personnel.

The Meeting concluded that the existing database on chlorfenapyr was adequate to characterize the potential hazards to fetuses, infants and children.

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) for chlorfenapyr of 0–0.03 mg/kg bw, based on a NOAEL of 2.8 mg/kg bw per day for decreases in body weight gain and vacuolation of the white matter of the brain at 16.6 mg/kg bw per day in an 18-month mouse study and a NOAEL of 2.9 mg/kg bw per day for reduced body weight and body weight gain and increased liver weight associated with hepatocellular enlargement at 15 mg/kg bw per day in a 2-year rat study. This was supported by a NOAEL of 2.6 mg/kg bw per day for reversible vacuolar myelinopathy, vacuolation and/or myelin sheath swelling of the brain and spinal cord in males at 13.6 mg/kg bw per day in a 1-year study of neurotoxicity in rats. A safety factor of 100 was applied.

The Meeting established an acute reference dose (ARfD) for chlorfenapyr of 0.03 mg/kg bw, based on the NOAEL of 3 mg/kg bw for depression of grooming and reactivity and decreased spontaneous motor activity observed at 10 mg/kg bw in a pharmacological study in mice. A 100-fold safety factor was applied.

Based on available information, it was not possible for the Meeting to determine whether the ADI and ARfD would also cover the metabolite AC 303,268.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen-month study of toxicity and carcinogenicity[a]	Toxicity	20 ppm, equal to 2.8 mg/kg bw per day	120 ppm, equal to 16.6 mg/kg bw per day
		Carcinogenicity	240 ppm, equal to 34.5 mg/kg bw per day[b]	—
	Pharmacological study[c]	Toxicity	3 mg/kg bw	10 mg/kg bw
Rat	Two-year study of toxicity and carcinogenicity[a]	Toxicity	60 ppm, equal to 2.9 mg/kg bw per day	300 ppm, equal to 15 mg/kg bw per day
		Carcinogenicity	600 ppm, equal to 30.8 mg/kg bw per day[b]	—
	Two-generation study of reproductive toxicity[a]	Reproductive toxicity	600 ppm, equal to 44 mg/kg bw per day[b]	—
		Parental toxicity	60 ppm, equal to 5 mg/kg bw per day	300 ppm, equal to 22 mg/kg bw per day
		Offspring toxicity	60 ppm, equal to 5 mg/kg bw per day	300 ppm, equal to 22 mg/kg bw per day
	Developmental toxicity study[c]	Maternal toxicity	75 mg/kg bw per day	225 mg/kg bw per day
		Embryo and fetal toxicity	225 mg/kg bw per day[b]	—
	Acute neurotoxicity study[c]	Toxicity	45 mg/kg bw	90 mg/kg bw
		Neurotoxicity	180 mg/kg bw[b]	—
	One-year neurotoxicity study[a]	Neurotoxicity	60 ppm, equal to 2.6 mg/kg bw per day	300 ppm, equal to 13.6 mg/kg bw per day
	Developmental neurotoxicity study[c]	Maternal toxicity	15 mg/kg bw per day[b]	—
		Offspring neurotoxicity	10 mg/kg bw per day	15 mg/kg bw per day
Rabbit	Developmental toxicity study[c]	Maternal toxicity	5 mg/kg bw per day	15 mg/kg bw per day
		Embryo and fetal toxicity	30 mg/kg bw per day[b]	—
Dog	Thirteen-week and 1-year studies of toxicity[a,d]	Toxicity	120 ppm, equal to 4 mg/kg bw per day	240 ppm, equal to 8.7 mg/kg bw per day

[a] Dietary administration.
[b] Highest dose tested.
[c] Gavage administration.
[d] Two or more studies combined.

Estimate of acceptable daily intake for humans

0–0.03 mg/kg bw

Estimate of acute reference dose

0.03 mg/kg bw

Information that would be useful for the continued evaluation of the compound

Additional studies on the toxicity of AC 303,268 to enable adequate characterization of the dietary risk from this metabolite. The Meeting was aware that additional studies on the compound have been performed but did not have access to sufficiently detailed reports to enable their evaluation.

Results from epidemiological, occupational health and other such observational studies of human exposure.

Critical end-points for setting guidance values for exposure to chlorfenapyr

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Slow, approximately 80% and 65% in the low-dose and high-dose groups, respectively
Dermal absorption	No data
Distribution	Extensive
Potential for accumulation	No evidence of accumulation
Rate and extent of excretion	Rapid and almost complete in 168 h in urine and faeces
Metabolism in animals	Absorbed dose metabolized by *N*-dealkylation, debromination, ring hydroxylation and conjugation
Toxicologically significant compounds in animals, plants and the environment	Chlorfenapyr, AC 303,268
Acute toxicity	
Rat, LD_{50}, oral	441 mg/kg bw
Rabbit, LD_{50}, dermal	> 2000 mg/kg bw
Rat, LC_{50}, inhalation	0.83 mg/l (4 h aerosol, whole-body exposure)
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Not irritating
Dermal sensitization	Not sensitizing (Magnusson & Kligman)
Short-term studies of toxicity	
Target/critical effect	Decreased body weight and weight gain, increased liver weights, vacuolation of the white matter (rat and mouse)
Lowest relevant oral NOAEL	4 mg/kg bw per day (dog)
Lowest relevant dermal NOAEL	100 mg/kg bw per day (rabbit)
Lowest relevant inhalation NOAEC	20 mg/m^3 (rat)
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Reduced growth rate and feed intake, vacuolation of the white matter (mice), haematological changes (rat)
Lowest relevant NOAEL	2.8 mg/kg bw per day (mouse carcinogenicity study)
Carcinogenicity	Not carcinogenic
Genotoxicity	
	Not genotoxic

Reproductive toxicity	
Reproduction target/critical effect	Reductions in pup body weights at parentally toxic doses
Lowest relevant parental NOAEL	5 mg/kg bw per day
Lowest relevant reproductive NOAEL	44 mg/kg bw per day (highest dose tested)
Lowest relevant offspring NOAEL	5 mg/kg bw per day
Developmental toxicity	
Developmental target/critical effect	Not teratogenic, no developmental toxicity
Lowest relevant maternal NOAEL	5 mg/kg bw per day (rabbit)
Lowest relevant developmental NOAEL	30 mg/kg bw per day (highest dose tested) (rabbit)
Neurotoxicity	
Acute neurotoxicity target/critical effect	Not acutely neurotoxic
One-year neurotoxicity target/critical effect	Vacuolation of the white matter (reversible) (rat)
Lowest relevant NOAEL	2.6 mg/kg bw per day (rat)
Neurodevelopmental toxicity target/critical effect	Vacuolation of white matter of the brain (reversible) (rat)
Lowest relevant NOAEL	10 mg/kg bw per day
Other toxicological studies	
Acute toxicity of metabolites	AC 312,094: rat LD_{50} > 5000 mg/kg bw
	AC 303,268: rat LD_{50} = 27 mg/kg bw
	AC 322,250: rat LD_{50} = 2500 mg/kg bw
	AC 325,195: rat LD_{50} = 776 mg/kg bw
Genotoxicity of metabolites	Not genotoxic
Medical data	
	No data available

Summary

	Value	Study	Safety factor
ADI	0–0.03 mg/kg bw	Eighteen-month (mouse) and 2-year (rat) studies of toxicity; 1-year neurotoxicity study (rat)	100
ARfD	0.03 mg/kg bw	Pharmacological study (mouse)	100

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DINOTEFURAN

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Explanation

Dinotefuran is the International Organization for Standardization (ISO)–approved common name for (*EZ*)-(*RS*)-1-methyl-2-nitro-3-(tetrahydro-3-furylmethyl)guanidine (International Union of Pure and Applied Chemistry) (Chemical Abstracts Service No. 165252-70-0), a novel neonicotinic insecticide used in various crops. Dinotefuran acts as an agonist at the insect nicotinic acetylcholine receptor and exhibits broad insecticidal activity via ingestion and contact.

Dinotefuran has not been evaluated previously by the Joint FAO/WHO Meeting on Pesticide Residues and was reviewed at the present Meeting at the request of the Codex Committee on Pesticide Residues.

All critical studies were certified as complying with good laboratory practice (GLP).

Evaluation for acceptable daily intake

Unless otherwise stated, the studies evaluated in this monograph were performed by laboratories that were certified for GLP and complied with the relevant Organisation for Economic Co-operation and Development (OECD) test guideline(s) or similar guidelines of the European Union (EU) or United States Environmental Protection Agency (USEPA). As these guidelines specify the clinical pathology tests normally performed and the tissues normally examined, only significant exceptions to these guidelines are reported here, to avoid repetitive listing of study parameters.

1. Biochemical aspects

1.1 Absorption, distribution and excretion

The absorption, distribution, metabolism and elimination (ADME) of ^{14}C-labelled dinotefuran (purity of non-radiolabelled test material: 98.92% and 99.47%; radiochemical purity: tetrahydrofuran-labelled > 98%, guanidine-labelled > 99%) were investigated in male and female Crl:CD(SD) BR rats and Hla(SD)CVF rats (bile duct–cannulated animals) (Cheng, 2000a,b, 2001). The study was conducted according to Japanese Ministry of Agriculture, Forestry, and Fisheries (JMAFF) test guideline 59 NohSan No. 4200. The test substances were suspended in 0.5% carboxymethylcellulose for oral administration or in 0.9% saline for intravenous administration. The animals were treated at a constant dose volume of 10 ml/kg body weight (bw), according to the schedule in Table 1.

In two preliminary groups treated with (^{14}C-tetrahydrofuran)-labelled (group P-1) or (^{14}C-guanidine)-labelled (group P-2) dinotefuran, the distribution of radioactivity between urine, faeces and carcass was similar, and radioactivity in expired air amounted to only 0.05% of the administered dose. Therefore, the definitive studies were performed with an approximate 1:1 ratio of both radiolabelled forms. Expired air was not collected in subsequent experiments. The schedule for sample collection and the terminal procedures are shown in Table 2.

There were no treatment-related clinical signs of toxicity, although one animal in group F died shortly after a difficult 4-hour blood sample.

Treatment of the two preliminary groups with tetrahydrofuran-labelled and guanidine-labelled dinotefuran demonstrated that the distribution of radioactivity between urine, faeces and carcass was similar and that radioactivity in expired air amounted to only 0.01–0.05% of the administered dose (Table 3).

In the main study, the total mean recovery of radioactivity ranged from 92.7% to 103% of the administered dose, with 87.7–99.8% recovered in urine, 1.06–3.16% recovered in faeces and 0.62–6.42% recovered from the cages. The similar, and extensive, recoveries in urine following oral and intravenous administration indicate almost complete absorption of ^{14}C-labelled dinotefuran from the gastrointestinal tract. Radioactivity was eliminated rapidly, with 84.3–98.9% of administered single doses excreted in urine within 24 hours. The absorption and route and rate of elimination were not influenced by sex, dose level or dose regimen (Table 4).

In bile duct–cannulated rats (groups K and L), a small amount of radioactivity (0.58–0.88% of the administered dose) was detected in bile samples after single doses of either 50 or 1000 mg/kg bw, indicating very limited enterohepatic recirculation of radioactivity (Table 5).

The mean maximum plasma concentration (C_{max}) of [^{14}C]dinotefuran ranged from 40.8 to 47.4 parts per million (ppm) at 0.25–0.625 hour after administration of single or repeated low oral doses (Table 6). A single high oral dose produced C_{max} values of 566 and 471 ppm in males and females, respectively, at approximately 2 hours after administration. The elimination half-life ranged from 3.64 to 16.1 hours for single and repeated low oral doses, respectively. A single high oral dose produced half-life values of 13.8 and 15.2 hours in males and females, respectively. Area under the plasma concentration–time curve (AUC) values following single and repeated low oral doses were in the range 69.0–110 ppm·h, compared with values of 2660 and 2360 ppm·h in males and females, respectively, after a single high oral dose. As the ratios of dose to AUC were comparable for the low

and high dose levels, absorption and pharmacokinetic characteristics of [^{14}C]dinotefuran were considered to be linear within the dose range 50–1000 mg/kg bw.

Table 1. Treatment schedule in the ADME study in rats

Group	Dose (mg/kg bw)	No. of doses	No. of males/females	Study element
P-1	50	1	3/3	Oral low dose—[F-^{14}C]dinotefuran[a]
P-2	50	1	3/3	Oral low dose—[G-^{14}C]dinotefuran[a]
A	50	1	5/5	Intravenous low dose
B	50	1	5/5	Oral low dose
C	50	14 + 1	5/5	Oral repeated low dose[b]
C-1	50	7	5/5	Oral repeated low dose[c]
D	1000	1	5/5	Oral high dose
E	50	1	5/5	PK—oral low dose
F	50	14 + 1	5/5	PK—oral repeated low dose[b]
F-1	50	7	5/5	PK—oral repeated low dose[c]
G	1000	1	5/5	PK—oral high dose
H	50	1	9/9	TD—oral low dose
I	50	14 + 1	9/9	TD—oral repeated low dose[b]
I-1	50	7	9/9	TD—oral repeated low dose[c]
J	1000	1	9/9	TD—oral high dose
K	50	1	4/4	BE—oral low dose
L	1000	1	4/4	BE—oral high dose
M	50	1	0/9	PT—oral low dose[d]
N	50	1	0/9	MT—oral low dose[e]
O	50	1	4/4	WBA—oral low dose
Q	1000	1	4/4	WBA—oral high dose
R	0	1	2/2	Vehicle control, oral

From Cheng (2000a)

BE, biliary excretion; [F-^{14}C]dinotefuran, [tetrahydrofuran-^{14}C]dinotefuran; [G-^{14}C]dinotefuran, [guanidine-^{14}C]dinotefuran; MT, milk transfer; PK, pharmacokinetic; PT, placental transfer; TD, tissue distribution; WBA, whole-body autoradiography (qualitative)

[a] Expired air collected to determine the radiolabelled material.
[b] Fourteen days of non-radiolabelled treatment followed by one radiolabelled dose.
[c] Seven days of radiolabelled treatment.
[d] Pregnant females at approximately day 18 of gestation.
[e] Lactating females approximately 12 days postpartum.

Table 2. Sample collection schedule and terminal procedures in the ADME study in rats

Group identity	Samples collected, collection schedule and terminal procedures
P-1, P-2	Expired air: 24 h intervals for 7 days
(oral low dose)	Urine/faeces: 0–6, 6–12, 12–24, 24–48, 48–72, 72–96, 96–120, 120–144, 144–168 h
	Whole blood/plasma: at termination
	Cage rinse: 0–24 h
	Cage wash/wipe: after final excreta collection
	Termination: after final excreta collection, carcasses retained

Table 2 (continued)

Group identity	Samples collected, collection schedule and terminal procedures
A, B, C, D	Urine/faeces: 0–6, 6–12, 12–24, 24–48, 48–72, 72–96, 96–120, 120–144, 144–168 h
(iv low dose)	Whole blood/plasma: at termination
(oral low dose)	Cage rinse: 0–24 h
(oral high dose)	Cage wash/wipe: after final excreta collection
(repeated low dose)	Termination: after final excreta collection, major organs/tissues + carcasses collected
C-1	Urine/faeces: 0–24, 24–48, 48–72, 72–96, 96–120, 120–144, 144–168 h until day 7; then 0–6, 6–12, 12–24, 24–48, 48–72, 72–96, 96–120, 120–144, 144–168 h
(repeated low dose—7 radioactive doses)	Whole blood/plasma: at termination
	Cage rinse: 24 h intervals
	Cage wash/wipe: after final excreta collection
	Termination: after final excreta collection, major organs/tissues + carcasses collected
E, F, F-1, G	Blood plasma: predose, 0.25, 0.5, 0.75, 1.0, 1.5, 2, 4, 8, 12, 24, 48 and 72 h
(PK)	Termination: after final collection, carcasses discarded
H, I, I-1	Whole blood and plasma: at termination
(TD—low dose + repeat)	Termination: 3 animals sacrificed at 0.5, 1.5 and 4 h after final dose, 30 major organs/tissues + carcasses collected
J	Whole blood/plasma: at termination
(TD—high dose)	Termination: 3 animals sacrificed at 1.5, 4 and 8 h after dose, 30 major organs/tissues + carcasses collected
K, L	Urine/faeces: 0–6, 6–12, 12–24, 24–48 h
(BE)	Whole blood/plasma: at termination
	Bile: 24 h predose, 0–6, 6–12, 12–24, 24–48 h
	Cage rinse: 0–24 h
	Cage wash/wipe: after final excreta collection
	Termination: after final excreta collection, major organs/tissues + carcasses collected
M	Whole blood/plasma: at termination
(PT)	Termination: 3 animals per time point sacrificed at 0.5, 1.5 and 4 h after dose, 2 whole fetuses per animal, selected tissues from 2 further fetuses per animal (placenta, amniotic fluid, blood, brain, heart, liver, lungs, kidneys, carcass), maternal tissues collected
N	Milk: 0.5, 1.5 and 4 h
(MT)	Whole blood/plasma: at termination
	Termination: 3 animals per time point sacrificed at 0.5, 1.5 and 4 h after milk collection, carcasses discarded
O (WBA—low dose)	Termination: 1 animal of each sex per time point sacrificed at 0.5, 1.5, 4 and 24 h post-dose and subjected to qualitative WBA
Q (WBA—high dose)	Termination: 1 animal of each sex per time point sacrificed at 1.5, 4, 8 and 72 h post-dose and subjected to qualitative WBA
R	Urine/faeces: 0–6, 6–12, 12–24, 24–48, 48–72, 72–96, 96–120, 120–144, 144–168 h
(vehicle control)	Whole blood/plasma: at termination
	Termination: after final excreta collection, carcasses retained

From Cheng (2000a)

BE, biliary excretion; iv, intravenous; MT, milk transfer; PK, pharmacokinetic; PT, placental transfer; TD, tissue distribution; WBA, whole-body autoradiography (qualitative)

Table 3. Recovery of radioactivity at 168 hours in the preliminary ADME study in rats

Sex	Dose (mg/kg bw); label	Recovery of radioactivity (% of administered dose)					
		Urine	Faeces	Cage	Expired air	Tissues and carcass	Total
Males	1 × 50; [F-^{14}C]	90.3	1.39	5.73	0.01	0.09	97.5
	1 × 50; [G-^{14}C]	90.6	4.02	2.97	0.05	0.10	97.8
Females	1 × 50; [F-^{14}C]	82.0	7.32	8.23	0.02	0.44	98.0
	1 × 50; [G-^{14}C]	93.5	1.22	2.51	0.05	0.08	97.3

From Cheng (2000a)
[F-^{14}C]dinotefuran, [tetrahydrofuran-^{14}C]dinotefuran; [G-^{14}C]dinotefuran, [guanidine-^{14}C]dinotefuran

Table 4. Recovery of radioactivity at 168 hours in the ADME study in rats

Sex	Dose (mg/kg bw); route	Recovery of radioactivity (% of administered dose)				
		Urine	Faeces	Cage	Tissues and carcass	Total
Males	1 × 50; intravenous	96.7	1.06	1.90	0.09	99.7
	1 × 50; oral	98.9	1.66	1.33	0.06	102
	15 × 50; oral	96.8	1.54	2.83	0.06	101
	7 × 50; oral	98.3	1.85	2.42	0.10	103
	1 × 1000; oral	90.1	2.15	2.52	0.10	94.7
Females	1 × 50; intravenous	96.6	1.26	1.42	0.05	99.2
	1 × 50; oral	99.8	1.19	0.62	0.08	102
	15 × 50; oral	89.7	3.16	6.42	0.21	99.3
	7 × 50; oral	95.8	1.53	4.88	0.10	102
	1 × 1000; oral	87.7	2.39	2.67	0.06	92.7

From Cheng (2000a)

Table 5. Recovery of radioactivity at 48 hours in bile duct–cannulated rats

Sex	Dose (mg/kg bw); route	Recovery of radioactivity (% of administered dose)					
		Urine	Faeces	Bile	Cage	Tissues and carcass	Total
Males	1 × 50; oral	94.7	1.08	0.62	2.70	0.39	99.5
	1 × 1000; oral	85.2	1.33	0.78	7.04	0.38	94.7
Females	1 × 50; oral	90.9	1.21	0.58	5.95	0.51	99.2
	1 × 1000; oral	90.3	1.34	0.88	3.83	2.43	98.8

From Cheng (2000a)

Radioactivity was widely distributed in all tissues examined 0.5 hour after a single oral dose of 50 mg/kg bw (Table 7). At this time, only the concentrations in the kidneys (79.4 ppm), stomach (67.3 ppm) and urinary bladder (45.8 ppm) were higher than those in plasma (40.6 ppm). Tissue concentrations declined quickly, and at 168 hours after dosing, concentrations in all tissues, with the exception of male skin (0.05 ppm), kidneys (0.01 ppm) and mammary gland (0.02 ppm), were below the limit of detection (0.001 ppm). All tissues were below the limit of detection 168 hours after a single intravenous dose. With the exceptions of male and female skin (0.007 and 0.014 ppm, respectively), female bone (0.004 ppm), female intestinal tract (0.003 ppm) and female mammary gland (0.018 ppm), all tissues were below the limit of detection 168 hours after 15 oral doses of 50 mg/kg bw. Radioactivity was widely distributed in most tissues examined following 7 daily oral doses of 50 mg/kg bw and after a single oral dose of 1000 mg/kg bw. In these groups, low concentrations

occurred in plasma (0.002–0.028 ppm), and the highest concentrations occurred in female mammary gland (0.324–0.703 ppm) and in the skin (0.193–0.692 ppm). The results indicate that the disposition of radioactivity is similar following single or multiple dosing regimens and after low or high doses.

Table 6. Toxicokinetic parameters calculated for [^{14}C]dinotefuran in rats

Sex	Dose (mg/kg bw); route	C_{max} (ppm)	T_{max} (h)	$t_{½}$ (h)	$AUC_{0–t}$ (ppm·h)	$AUC_{0–\infty}$ (ppm·h)
Males	1 × 50; oral	40.8	0.50	3.64	83.3	83.3
	15 × 50; oral	47.4	0.45	5.65	92.1	92.1
	7 × 50; oral	41.5	0.63	6.28	91.2	91.2
	1 × 1000; oral	566	2.10	13.8	2660	2660
Females	1 × 50; oral	45.6	0.25	7.86	110	110
	15 × 50; oral	42.2	0.38	6.89	76.0	76.0
	7 × 50; oral	43.8	0.31	16.1	69.0	69.2
	1 × 1000; oral	471	2.00	15.2	2360	2370

From Cheng (2000a)
AUC, area under the plasma concentration–time curve; C_{max}, maximum concentration in plasma; $t_{½}$, half-life; T_{max}, time to reach C_{max}

In pregnant rats (group M), radioactivity was rapidly transferred to fetuses and rapidly distributed to the fetal tissues. Maximum fetal concentrations occurred in all tissues examined (except fetal brain) within 0.5 hour of maternal treatment (Table 8). Subsequently, radioactivity declined rapidly to low levels within 4 hours. Similar concentrations occurred in maternal and fetal blood, suggesting a rapid equilibration and similar tissue distribution in maternal and fetal tissues.

In lactating rats (group N), radioactivity was rapidly transferred from maternal blood to the milk of lactating animals at day 12 postpartum. C_{max} values for maternal plasma and milk were 29.3 and 34.8 ppm, respectively, 0.5 hour post-dosing (Table 9). Concentrations in milk declined rapidly to 6.51 ppm after 4 hours. Calculation of pharmacokinetic parameters gave an elimination half-life of 1.39 hours in milk, indicating that within 14 hours of administration, the expected concentration of radioactivity would be lower than the limit of detection (0.002 ppm).

Whole-body autoradiography after single oral doses of 50 or 1000 mg/kg bw (groups O and Q) were consistent with the results obtained for the tissue distribution groups mentioned previously. Tissue radioactivity derived from [^{14}C]dinotefuran was widely distributed and highest at the first sampling interval, 0.5 hour and 1.5 hours, for 50 and 1000 mg/kg bw, respectively. Thereafter, levels of radioactivity were declining 1.5 hours after administration of 50 mg/kg bw and 8 hours after administration of 1000 mg/kg bw. Elimination was almost complete after 24 hours (50 mg/kg bw) and 72 hours (1000 mg/kg bw); no detectable radioactivity was apparent in either sex. The highest levels of radioactivity were generally found in the urine, followed by the gastrointestinal contents and renal medulla. Low levels of radioactivity were detected in the brain and gonads of both sexes after 50 or 1000 mg/kg bw. There were no apparent sex-related or dose-related differences in the overall distribution trends (Cheng, 2000a).

Table 7. Concentrations of radioactivity in organs/tissues of rats after a single oral dose of 50 mg/kg bw

Tissue	Concentration of radioactivity (ppm)							
	Males[a]				Females[a]			
	0.5 h	1.5 h	4 h	168 h	0.5 h	1.5 h	4 h	168 h
Blood	34.8	13.5	1.92	ND	35.0	12.5	1.40	ND
Plasma	40.6	15.9	2.24	ND	41.4	14.6	1.64	ND
Eyes	14.8	8.88	2.16	ND	13.0	8.75	1.49	ND
Skin	33.9	15.1	2.27	0.05	29.8	11.6	1.50	ND
Mammary gland	NA	NA	NA	NA	26.7	10.9	1.23	0.02
Urinary bladder	45.8	95.3	5.9	ND	32.4	19.0	6.31	ND
Fat (reproductive)	7.88	2.36	0.44	ND	5.31	1.97	0.30	ND
Prostate	32.3	21.8	4.41	ND	NA	NA	NA	NA
Testes	18.5	15.8	3.03	ND	NA	NA	NA	NA
Ovaries	NA	NA	NA	NA	28.0	10.6	1.16	ND
Uterus	NA	NA	NA	NA	33.5	13.1	1.18	ND
Spleen	28.1	10.4	1.3	ND	28.1	9.83	1.07	ND
Adrenals	30.9	12.7	2.04	ND	29.9	12.9	1.81	ND
Kidneys	79.4	33.5	3.98	0.01	72.4	28.9	3.90	ND
Liver	36.3	13.9	2.11	ND	37.6	12.0	1.54	ND
Thymus	32.6	12.3	1.95	ND	32.6	12.0	1.37	ND
Heart	29.6	11.4	1.71	ND	26.0	11.0	1.24	ND
Lungs	32.9	12.4	1.99	ND	34.5	12.3	1.40	ND
Thyroid/parathyroid	24.5	9.99	1.56	ND	27.7	10.7	1.24	ND
Brain	2.92	2.06	0.32	ND	2.24	1.90	0.41	ND
Pituitary gland	31.3	12.0	1.83	ND	32.6	11.7	1.19	ND
Muscle (thigh)	31.4	12.4	2.13	ND	29.5	13.0	1.61	ND
Bone marrow	29.9	10.3	1.82	ND	28.6	9.96	1.81	ND
Bone (femur)	16.2	6.19	1.51	ND	11.9	4.98	1.06	ND
Pancreas	28.1	10.7	1.83	ND	29.0	10.1	1.50	ND
Lymph node	29.2	10.4	1.73	ND	28.1	9.61	1.35	ND
Stomach contents[b]	14.9	5.00	0.08	ND	28.9	1.46	0.67	ND
Intestinal tract contents[b]	8.28	7.26	4.12	ND	9.78	5.48	4.00	ND
Stomach	67.3	27.5	2.39	ND	171	15.5	5.20	ND
Intestinal tract	34.3	24.5	4.1	ND	47.5	18.2	4.15	ND

From Cheng (2000a)
NA, not applicable; ND, not detected
[a] Group H: 0.5-hour, 1.5-hour and 4-hour time points; Group B: 168-hour time point.
[b] Includes wash.

Table 8. Concentrations of radioactivity in pregnant rats (day 18 post-coitum) after a single oral dose of 50 mg/kg bw

Tissue	Concentration of radioactivity (ppm)		
	0.5 h	1.5 h	4 h
Fetal blood	23.9	17.3	3.63
Fetal brain	16.6	17.8	4.21
Fetal heart	27.1	18.2	3.74
Fetal kidneys	25.8	15.0	3.90
Fetal liver	18.9	11.8	2.62
Fetal lungs	23.1	16.7	3.41
Fetus	23.5	17.2	3.90
Maternal blood	38.1	18.5	3.99
Maternal plasma	44.3	21.3	4.65

From Cheng (2000a)

Table 9. Concentrations of radioactivity in lactating rats (day 12 postpartum) after a single oral dose of 50 mg/kg bw

Tissue	Concentration of radioactivity (ppm)		
	0.5 h	1.5 h	4 h
Blood	24.8	14.3	2.87
Plasma	29.3	17.2	3.48
Milk	34.8	28.1	6.51

From Cheng (2000a)

In a special study in neonatal (i.e. 12-day-old) rats, the ADME of (^{14}C-guanidine)-labelled dinotefuran (purity 99.47%; radiochemical purity 99.6%) were investigated in 28 male and 28 female Crl:CD(SD) BR rats (Cheng, 2000c,d). The study was conducted according to JMAFF test guideline 59 NohSan No. 4200. The test substance was administered as a suspension in 0.5% carboxymethylcellulose by oral gavage at a single dose of 50 mg/kg bw using a dose volume of 10 ml/kg bw. For analysis of excreta, blood and selected tissues, 25 pups of each sex were used (group 1), whereas 3 pups of each sex (group 2) were used for whole-body autoradiographic analysis. Morbidity and mortality checks were performed twice daily, clinical signs were recorded daily and body weights were recorded on the day of treatment. Urine and faeces were collected together as excreta from group 1 animals. After each sampling interval, the pups and cages were wiped with gauze pads and the pads added to the excreta. Excreta samples were pooled to form a single sample for each sex and sampling interval. Five pups of each sex from group 1 were killed at 0.5 or 1.5 hours post-dosing, and 15 pups of each sex at 4 hours post-dosing. Blood samples were collected and pooled to form a single sample for each sex and sampling interval. An aliquot of each blood sample was retained for radioanalysis, and the residue was centrifuged to separate plasma and cellular components. The cellular component was discarded. Liver, kidneys, intestinal tract and contents, stomach, stomach contents and residual carcass were excised, rinsed, blotted dry and weighed, and each tissue was pooled to form a single sample for each sex and sampling interval. The weight of each pooled sample was recorded. One pup of each sex from group 2 was killed at 0.5, 1.5 and 4 hours post-dosing, and the carcasses were prepared for whole-body autoradiography.

Radioactivity was rapidly and extensively absorbed, widely distributed and rapidly eliminated from all tissues sampled. The mean total recovery of radioactivity from both sexes of pups was within the range 87.6–98.2% for all sampling intervals (Table 10). The recovery of radioactivity at 4 hours post-dosing amounted to 36.3% and 31.8% in excreta, 22.2% and 20.7% in stomach contents and

30.6% and 31.8% in the residual carcass in males and females, respectively. Absorption from the gastrointestinal tract amounted to at least 75% within 4 hours in both sexes.

Table 10. Recovery of radioactivity in neonatal rats after a single oral dose of 50 mg/kg bw

Tissue	Recovery of radioactivity (% of administered dose)					
	Males			Females		
	0.5 h	1.5 h	4 h	0.5 h	1.5 h	4 h
Blood	1.84	1.38	0.69	1.42	1.26	0.53
Excreta	3.23	22.1	36.3	5.75	26.3	31.8
Intestinal tract + contents	2.91	2.28	1.80	3.37	2.25	1.42
Kidneys	0.95	0.28	1.15	0.84	0.89	0.59
Liver	1.47	1.05	0.64	1.43	0.97	0.52
Residual carcass	31.5	34.3	30.6	25.1	26.1	31.8
Stomach	1.14	0.45	0.88	1.93	0.31	0.18
Stomach contents	52.2	36.4	22.2	54.1	36.0	20.7
Total	95.3	98.2	94.2	94.0	94.1	87.6

From Cheng (2000c)

The maximum plasma concentration of radioactivity in both sexes was approximately 21 ppm and occurred at 0.5 hour post-dosing (Table 11). Thereafter, it declined to approximately 9 ppm at 4 hours post-dosing. Concentrations in the stomach and kidneys were higher than those in plasma at all sampling intervals, but were comparable to those in liver and plasma. Concentrations in blood and plasma were also comparable, indicating that radioactivity was not associated with the cellular elements of blood. There were no apparent sex-related differences in the absorption or elimination of radioactivity.

Table 11. Concentrations of radioactivity in neonatal rats after a single oral dose of 50 mg/kg bw

Tissue	Concentration of radioactivity (ppm)					
	Males			Females		
	0.5 h	1.5 h	4 h	0.5 h	1.5 h	4 h
Blood	20.3	18.2	9.06	17.0	15.4	6.97
Intestinal tract + contents	28.8	21.8	14.2	28.3	20.2	12.3
Kidneys	36.1	10.2	45.8	30.9	34.3	22.6
Liver	23.7	15.1	10.2	21.3	15.1	7.66
Plasma	21.3	19.1	9.42	21.2	18.9	8.71
Residual carcass	18.7	20.5	17.9	15.3	16.1	19.5
Stomach	76.1	33.7	59.9	110	23.2	13.7
Stomach contents	70.8	48.3	33.4	78.1	38.4	32.7

From Cheng (2000c)

Autoradiographic data indicated that radioactivity derived from (^{14}C-guanidine)-labelled dinotefuran was widely distributed throughout the organs and tissues in male and female pups (Table 12). Most of the radioactivity was contained in the stomach and contents, kidneys, urinary bladder and urine. Maximum tissue concentrations occurred either 0.5 or 1.5 hours post-dosing. Overall, the concentration of radioactivity in most tissues declined during the 4 hours post-dosing. In contrast, the

concentrations in the renal medulla and cortex, the urinary bladder and urine increased from 0.5 to 4 hours post-dosing, indicating predominantly urinary elimination. Elimination from most tissues was incomplete at 4 hours post-dosing. Low levels of radioactivity occurred in the brain. There were no apparent sex differences in the tissue distribution of radioactivity.

Table 12. Concentrations of radioactivity in neonatal rats after a single oral dose of 50 mg/kg bw determined by quantitative whole-body autoradiography

Tissue	Concentration of radioactivity (ppm)					
	Males			Females		
	0.5 h	1.5 h	4 h	0.5 h	1.5 h	4 h
Adrenal gland	33.6	38.1	18.3	30.1	29.1	19.3
Aorta	27.9	23.2	7.86	25.5	16.8	11.9
Axillary lymph node	17.8	18.4	6.45	16.9	NR	8.75
Blood	15.9	17.2	5.70	19.5	13.5	8.10
Bone	12.6	11.8	4.77	14.2	10.4	7.05
Bone marrow	15.0	15.3	5.48	17.9	12.3	7.44
Cerebellum	9.00	15.3	5.66	10.5	12.6	8.53
Cerebrum	7.32	13.9	6.04	8.62	12.0	8.40
Cervical lymph node	22.7	17.9	7.66	16.5	12.6	9.67
Diaphragm	13.7	27.3	9.88	30.7	18.8	13.1
Epididymis	25.6	14.9	6.47	—	—	—
Exorbital lacrimal gland	21.5	24.1	9.69	21.8	14.3	12.6
Eye	10.7	14.2	7.14	13.0	11.8	10.7
Fat (abdominal)	5.74	8.58	5.66	7.83	2.82	2.48
Fat (brown)	13.0	13.2	5.59	17.8	11.1	7.82
Gastrointestinal tract	36.8	21.7	12.1	36.3	21.1	23.1
Gastrointestinal tract contents	22.7	25.0	16.7	29.7	18.6	28.6
Harderian gland	17.7	21.4	8.71	20.8	16.5	10.7
Inguinal lymph node	11.8	14.2	5.49	13.6	6.63	7.48
Intraorbital lacrimal gland	NR	22.3	8.07	22.6	17.2	7.51
Kidney	71.1	389	172	84.4	61.2	96.9
Liver	31.8	24.7	12.1	33.0	21.5	14.0
Lung	20.7	17.4	7.04	21.7	14.3	9.03
Medulla	6.26	13.2	5.63	7.22	11.4	8.14
Mesenteric lymph node	32.6	23.6	7.13	30.1	19.9	10.3
Muscle	18.7	19.1	7.27	19.5	14.3	9.05
Myocardium	23.3	21.4	7.83	26.5	17.1	10.4
Nasal turbinates	11.6	17.3	6.20	16.9	15.2	8.71
Oesophageal contents	58.4	19.2	NR	15.2	29.2	NR
Oesophagus	86.7	27.6	NR	29.4	14.4	10.5
Olfactory lobe	9.01	14.0	5.43	11.8	12.5	8.82
Ovary	—	—	—	28.8	16.3	14.2
Pancreas	28.8	26.2	19.6	34.2	24.7	21.4
Pineal gland	NR	20.6	NR	NR	NR	9.10

Tissue	Concentration of radioactivity (ppm)					
	Males			Females		
	0.5 h	1.5 h	4 h	0.5 h	1.5 h	4 h
Pituitary gland	13.1	14.9	6.75	15.5	12.3	9.96
Popliteal lymph node	18.1	10.8	NR	15.3	10.0	5.33
Preputial gland	NR	NR	21.5	47.5	NR	NR
Renal cortex	62.5	138	137	78.7	52.3	76.5
Renal medulla	83.5	554	231	94.2	74.6	153
Salivary gland	18.9	21.7	7.82	23.5	16.3	10.2
Seminal vesicle	16.2	24.9	15.7	—	—	—
Skin	18.4	23.0	9.03	18.9	17.4	13.0
Spinal cord	8.32	12.3	4.56	9.86	10.2	6.21
Spleen	26.0	26.1	11.4	29.4	19.4	14.6
Stomach	52.0	40.7	35.1	74.9	48.0	35.5
Stomach contents	222	238	261	517	328	296
Testes	22.6	24.7	19.3	—	—	—
Thymus	18.3	18.0	7.39	19.9	14.3	9.00
Thyroid gland	NR	NR	8.12	21.5	15.9	10.2
Trachea	NR	16.1	NR	21.7	38.4	NR
Urinary bladder	64.9	111	1420	222	653	1240
Urine	45.0	164	291	35.6	166	338
Uterus	—	—	—	19.1	11.0	18.6

From Cheng (2000c)
NR, not represented (tissue not present in section)

The gastrointestinal tract and contents of neonatal pups contained 56–60% of the administered radioactivity 0.5 hour post-dosing, in contrast to 10–15% in adults (Table 13). Concentrations had declined by 4 hours post-dosing to 22–25% of the administered dose in pups and to less than 3% in adults. Thus, at 0.5 hour, oral absorption in pups amounted to approximately 50%, compared with approximately 90% in adults. The kidneys of pups contained less radioactivity than adult kidneys at 0.5 hour and more after 4 hours. These data also support the contention that oral absorption and urinary elimination are slower in neonatal pups than in the adult animal.

The concentration of plasma radioactivity in neonatal rats was approximately 52% of that of the adult rat 0.5 hour post-dosing, whereas at 4 hours, the neonatal plasma concentration was more than 4 times the adult concentration (Table 14). A similar pattern was evident in liver. The concentrations in the kidneys and stomach contents of neonatal pups were markedly higher than in adults at 4 hours post-dosing. These data indicate that oral absorption and urinary elimination in neonates are slower than in the adult animal. Possible reasons for rate differences include incomplete development of the gastrointestinal tract and kidneys in the neonate, the absence of the maternal stimulus for neonatal micturition and the presence of feed in the gut.

1.2 Biotransformation

In the ADME study of ^{14}C-labelled dinotefuran described above (Cheng, 2000a,b, 2001), pooled tissue, fluid and excreta samples for metabolite identification were analysed for radioactivity either by direct liquid scintillation counting (LSC) or by combustion and LSC. With the exception of urine and bile samples, pooled samples were sequentially extracted, and samples containing large amounts of radioactivity were further processed by solid-phase extraction (SPE). The total radioactive content of each fraction was determined by LSC. Samples with sufficient radioactivity for further

analysis were subjected to high-performance liquid chromatography (HPLC) to determine metabolite profiles by comparison with reference standards. Urine was also analysed by two-dimensional thin-layer chromatography. Urine and faecal samples were used for isolation/fractionation of radioactive metabolites by sequential SPE. Selected column eluates were subjected to analysis by liquid chromatography with mass spectrometry or tandem mass spectrometry to obtain spectral data from the isolated metabolites.

Table 13. Recovery of radioactivity in neonatal and adult rats after a single oral dose of 50 mg/kg bw

Sex	Tissue	Recovery of radioactivity (% of administered dose)					
		0.5 h		1.5 h		4 h	
		Neonate	Adult	Neonate	Adult	Neonate	Adult
Males	Blood	1.84	2.70	1.38	1.07	0.69	0.13
	Kidneys	0.95	1.55	0.28	0.65	1.15	0.08
	Liver	1.47	2.98	1.05	1.10	0.64	0.17
	Stomach contents	52.2	2.41	36.4	0.64	22.2	0.02
	Stomach	1.14	0.88	0.45	0.34	0.88	0.03
	Intestine + contents	2.91	7.62	2.28	4.48	1.80	2.75
Females	Blood	1.42	2.69	1.26	0.88	0.53	0.09
	Kidneys	0.84	1.21	0.89	0.46	0.59	0.06
	Liver	1.43	2.41	0.97	0.79	0.52	0.10
	Stomach contents	54.1	4.24	36.0	0.19	20.7	0.14
	Stomach	1.93	1.88	0.31	0.16	0.18	0.05
	Intestine + contents	3.37	8.24	2.25	3.86	1.42	2.17

From Cheng (2000a,c)

Table 14. Comparison of radioactivity concentrations in neonatal and adult rats after a single oral dose of 50 mg/kg bw

Sex	Tissue	Concentration of radioactivity (ppm)					
		0.5 h		1.5 h		4 h	
		Neonate	Adult	Neonate	Adult	Neonate	Adult
Males	Blood	20.3	34.8	18.2	13.5	9.06	1.92
	Plasma	21.3	40.6	19.1	15.9	9.42	2.24
	Kidneys	36.1	79.4	10.2	33.5	45.8	3.98
	Liver	23.7	36.3	15.1	13.9	10.2	2.11
	Stomach	76.1	67.3	33.7	27.5	59.9	2.39
	Stomach contents	70.8	14.9	48.3	5.00	33.4	0.083
Females	Blood	17.0	35.0	15.4	12.5	6.97	1.40
	Plasma	21.2	41.4	18.9	14.6	8.71	1.64
	Kidneys	30.9	72.4	34.3	28.9	22.6	3.90
	Liver	21.3	37.6	15.1	12.0	7.66	1.54
	Stomach	110	171	23.2	15.5	13.7	5.20
	Stomach contents	78.1	28.9	38.4	1.46	32.7	0.667

From Cheng (2000a,c)

Unchanged dinotefuran in urine accounted for 92.5–97.2% of total urine radioactivity (Table 15). A group of urinary metabolites, PHP and isomers, 446-DO and isomers, 446-CO, 446-DO-Ac, 446-OH-Ac and 446-OH+COOH, represented 2.08–5.95% of the administered radioactivity (see Appendix 1). Other minor urine metabolites, UF, FNG and DN, each accounted for no more than 0.53% of the administered radioactivity. Trace amounts (< 0.1% of the administered radioactivity) of nine other metabolites were also detected in urine.

Table 15. Detected metabolites of dinotefuran in urine in rats

Dose (mg/kg bw)	% of radioactivity in sample												
	Parent	F1[a]	F2[b]	F3[c]	UF	FNG	F4[d]	F5[e]	DN-2-OH	DN-3-OH[f]	BCDN	DN	446-NH_2
Males													
1 × 50, iv	94.4	0.03	2.18	2.31	0.32	0.48	0.05	0.10	0.02	0.00	0.02	0.04	0.00
1 × 50, oral	92.5	0.16	2.80	3.47	0.15	0.56	0.00	0.16	0.08	0.00	0.05	0.03	0.03
15 × 50, oral	94.0	0.14	2.48	2.53	0.21	0.37	0.07	0.00	0.00	0.09	0.00	0.13	0.00
7 × 50, oral	95.0	0.10	0.90	2.38	0.18	0.27	0.00	0.00	0.00	0.06	0.00	0.02	0.02
1 × 1000, oral	92.8	0.17	2.48	3.34	0.29	0.49	0.10	0.14	0.05	0.04	0.00	0.04	0.03
Females													
1 × 50, iv	97.2	0.10	1.07	1.33	0.13	0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1 × 50, oral	95.3	0.07	1.71	2.19	0.17	0.30	0.00	0.09	0.03	0.00	0.00	0.09	0.03
15 × 50, oral	95.3	0.18	1.71	2.07	0.16	0.39	0.00	0.07	0.00	0.07	0.00	0.08	0.00
7 × 50, oral	95.8	0.19	1.63	1.65	0.12	0.32	0.00	0.11	0.00	0.00	0.00	0.13	0.00
1 × 1000, oral	95.8	0.12	1.36	1.90	0.21	0.27	0.17	0.09	0.00	0.08	0.01	0.03	0.00

From Cheng (2000a)
iv, intravenous; RT, retention time
[a] Fraction 1 (RT: 8.2–9.7 min): mixture of MNG, 446-DO-Ac.
[b] Fraction 2 (RT: 12.0–14.0 min): mixture of PHPs, UF-DM, 446-OH+COOH.
[c] Fraction 3 (RT: 14.7–16.8 min): mixture of 446-CO, 446-DO, PHP-Ac.
[d] Fraction 4 (RT: 21.1–22.4 min).
[e] Fraction 5 (RT: 26.7–33.9 min): mixture of MG, MG-Ac.
[f] FNG-DN with an RT between that of DN-3-OH and BCDN also detected in some samples.

Unchanged dinotefuran was the major component of faecal radioactivity, but numerous minor metabolites were identified, and 0.01–1.75% of the administered radioactivity represented unidentified polar metabolites. Unchanged dinotefuran was also the major component of bile radioactivity, and minor metabolites were similar to those found in urine and faeces. Unchanged dinotefuran was the major component of plasma radioactivity (> 80%), with possible metabolites of MNG, 446-DO-Ac and PHPs. Overall, more than 90% of the radioactivity derived from [^{14}C]dinotefuran is excreted as unchanged parent compound following oral or intravenous administration.

In conclusion, dinotefuran was the major radioactive component in most tissues examined, and less than 10% of dinotefuran was metabolized. There were no apparent differences related to treatment regimen or sex in the metabolic handling of dinotefuran. Initially, enzymatic hydroxylation on the tetrahydrofuran ring occurs to form PHP isomers, followed by further oxidation, reduction and acetylation of PHP to produce possible isomers of 446-CO, 446-DO, PHP-Ac and 446-OH+COOH. Other routes of metabolism involve desmethylation to FNG, nitro-reduction to 446-NH_2 and further hydrolysis to DN and UF. The combination of these reactions at certain stages produced numerous

metabolites, such as UF-DM, FNG-DN, BCDN, DN-OH and isomers and DN-CO. Trace amounts of MNG, MG and MG-Ac indicate a small degree of cleavage at the C–N bond to yield the furan and guanidine moieties. A proposed metabolic pathway and the structures of the putative metabolites are shown in Figure 1.

In the special ADME study in neonatal (i.e. 12-day-old) rats described above (Cheng, 2000c,d), pooled samples of liver, kidneys, stomach, plasma, excreta and intestinal tract with contents collected 4 hours post-dosing from group 1 pups were extracted. The extracts were analysed by LSC to determine radioactivity recovery and by comparative HPLC to determine metabolite profiles. ^{14}C-UF and ^{14}C-DN were used for co-chromatography with test sample extracts.

The recovery of radioactivity from all matrices for metabolite profiling ranged from 96.6% to 100%. In all matrices, [^{14}C-guanidine]dinotefuran was the major component and accounted for 97.0–100% of the radioactivity in plasma, kidneys, stomach and excreta (Table 16). In the liver, [^{14}C-guanidine]dinotefuran accounted for 61.1% (males) and 66.5% (females) of radioactivity, and in the intestinal tract with contents, it accounted for 83.3% (males) and 76.3% (females). Putative metabolites in the neonate are shown in the legend to Table 16.

Metabolism in the neonatal rat was limited, and the metabolite profiles were comparable to those of the young adult rat (Cheng, 2000a), but fewer metabolites were detected in neonates (Table 17). These data suggest slower metabolism of [^{14}C-guanidine]dinotefuran, possibly due to incomplete development of neonatal liver function.

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

The results of acute toxicity studies with dinotefuran are summarized in Table 18.

In an acute oral toxicity study conducted according to OECD Test Guideline No. 401, male and female Crl:CD[SD]BR (SPF) rats received dinotefuran (purity 96.5%) in 0.5% aqueous methylcellulose. Initially, a dose range–finding study was performed comprising four groups of one animal of each sex treated with a dose level of 500, 1000, 3000 or 5000 mg/kg bw at a volume of 20 ml/kg bw. In phase I of the main study, five animals of each sex were treated at a volume of 10 ml/kg bw with 5000 mg/kg bw, and two groups of females only were treated at 3000 or 1000 mg/kg bw. In phase II of the main study, groups of five male animals were treated with a dose level of 1000, 2000, 3000 or 5000 mg/kg bw, and groups of five females were treated with a dose level of 1000, 2000, 3000 or 4000 mg/kg bw, each at a volume of 20 ml/kg bw. The main study animals were checked twice daily for morbidity or mortality. Clinical signs were recorded frequently on the day of treatment (main study animals) and then daily for 14 days. Body weights were recorded predosing and on days 7 and 14 or on the day of death. Decedents and survivors from the dose range–finding study were discarded without necropsy. Animals dying during the observation period and all survivors from the main study were submitted for necropsy and abbreviated postmortem examination.

In the range-finding study, the females treated at 3000 or 5000 mg/kg bw died on day 1; all other animals survived the observation period (Table 19). In phase I of the main study, there were no deaths at any dose level administered at a treatment volume of 10 ml/kg bw. The median lethal dose (LD_{50}) for dinotefuran administered at 10 ml/kg bw was greater than 5000 mg/kg bw. In phase II of the main study, deaths occurred in females treated at and above 2000 mg/kg bw and in males treated at and above 3000 mg/kg bw. All deaths in phase II occurred on the day of dosing or on the day following dosing.

Figure 1. Proposed metabolic scheme for dinotefuran (MTI-446) in rats

MTI-446

FNG

UF

UF-DM

FNG-DN

DN

446-NH2

DN-2-OH

BCDN

DN-DO

446-DO

446-DO-Ac

446-NU

PHP

PHP-Ac

DN-3-OH

DN-CO

MG

MG-Ac

MNG

446-OH+COOH

446-CO

1.oxidation
2.reduction
3.conjugation
4.re-cyclizaion
5.hydrolysis

equilibrium

Table 16. Detected metabolites of dinotefuran in neonatal rats after a single oral dose of 50 mg/kg bw

Tissue	% of radioactivity in sample / ppm					
	Dinotefuran	Fraction 1[a]	Fraction 2[b]	Fraction 3[c]	Fraction 4[d]	1+2+3+4
Males						
Liver	61.1/6.23	6.69/0.69	4.89/0.50	19.5/1.99	7.82/0.80	38.9/3.98
Kidneys	97.1/44.5	1.62/0.74	0.73/0.34	0.53/0.24	0.00/0.00	2.88/1.32
Stomach	99.0/59.3	0.21/0.13	0.78/0.47	0.00/0.00	0.00/0.00	0.99/0.60
ITC	83.3/11.8	0.00/0.00	15.4/2.19	1.40/0.20	0.00/0.00	16.8/2.39
Excreta	98.5/NA	1.49/NA	0.00/NA	0.00/NA	0.00/NA	1.49/NA
Plasma	100/9.42	0.00/0.00	0.00/0.00	0.00/0.00	0.00/0.00	0.00/0.00
Females						
Liver	66.5/5.09	5.17/0.39	3.76/0.29	23.0/1.76	1.62/0.12	33.6/2.56
Kidneys	97.0/21.9	2.28/0.52	0.68/0.15	0.00/0.00	0.00/0.00	2.96/0.67
Stomach	100/13.7	0.00/0.00	0.00/0.00	0.00/0.00	0.00/0.00	0.00/0.00
ITC	76.3/9.38	21.6/2.65	0.00/0.00	2.16/0.26	0.00/0.00	23.8/2.91
Excreta	100/NA	0.00/NA	0.00/NA	0.00/NA	0.00/NA	0.00/NA
Plasma	100/8.71	0.00/0.00	0.00/0.00	0.00/0.00	0.00/0.00	0.00/0.00

From Cheng (2000c)

ITC, intestinal tract with contents; NA, not calculated, because the net sample weight without cage wash was not known; RT, retention time

[a] Fraction 1 (RT: 4.5–9.4 min): Possible mixture of MNG, 446-DO-Ac and others.

[b] Fraction 2 (RT: 12.1–19.8 min): Possible mixture of PHPs, 446-DO, 446-CO, 446-OH+COOH, UF-DM, PHP-Ac, UF, FNG.

[c] Fraction 3 (RT: 38.1–44.0 min): Possible mixture of MG, MG-Ac, DN-DO, DN-OHs, DN-CO, BCDN, DN.

[d] Fraction 4 (RT: 46.8–47.0 min): Unknown.

Table 17. Comparison of extent of metabolism of dinotefuran in neonatal and adult rats

Matrix	% of radioactivity in sample							
	Males				Females			
	Neonate		Adult		Neonate		Adult	
	Parent	Metabolites	Parent	Metabolites	Parent	Metabolites	Parent	Metabolites
Plasma	100	0.00	87.1	6.80	100	0.00	NA	NA
Liver	61.1	38.9	4.66	87.0	66.5	33.5	0.00	92.9
Kidneys	97.1	2.88	62.6	21.1	97.0	2.96	72.2	16.7
Stomach	99.0	0.99	56.7	43.3	100	0.00	NA	NA
Excreta	98.5	1.49	88.2[a]	8.09[a]	100	0.00	93.1[a]	5.23[a]
ITC[a]	83.3	16.8	NS	NS	76.3	23.7	NS	NS

From Cheng (2000a) for neonatal rats, Cheng (2000c) for adult rats

ITC, intestinal tract with contents; NA, data not available (low radioactivity in sample); NS, no sample with contents

[a] Calculated by combining data from urine and faeces.

Table 18. Summary of acute toxicity studies with dinotefuran

Species	Strain	Sex	Route	Purity (%)	LD_{50} / LC_{50}	Reference
Rat	Crl:CD[SD]BR	M+F	Oral	96.5	2450 mg/kg bw	Glaza (1997a)
Mouse	Crl:CD-1[ICR]BR	M+F	Oral	96.5	2371 mg/kg bw	Glaza (1997b)
Rat	Crl:CD[SD]BR	M+F	Dermal	96.5	> 2000 mg/kg bw	Glaza (1997c)
Rat	Crl:WI[Glx/BRL/Han]BR	M+F	Inhalation	93.0	> 4.09 mg/l	Shepherd (1999)

F, female; LC_{50}, median lethal concentration; LD_{50}, median lethal dose; M, male

Table 19. Results of the acute oral toxicity study in rats

Dose (mg/kg bw)	No. of animals dying/no. tested					
	Range-finding study (dose volume 20 ml/kg bw)		Main study – phase I (dose volume 10 ml/kg bw)		Main study – phase II (dose volume 20 ml/kg bw)	
	Males	Females	Males	Females	Males	Females
500	0/1	0/1	—	—	—	—
1000	0/1	0/1	—	0/5	0/5	0/5
2000	—	—	—	—	0/5	3/5
3000	0/1	1/1	—	0/5	3/5	4/5
4000	—	—	—	—	—	5/5
5000	0/1	1/1	0/5	0/5	—	—

From Glaza (1997a)

In phase I of the main study, two females at 5000 mg/kg bw showed transient staggering gait on the day of treatment only and red staining of the face persisting for up to 3 days. One female treated at 3000 mg/kg bw also showed transient staggering gait on the day of treatment. A male at 5000 mg/kg bw showed transient excessive salivation, and a female at 1000 mg/kg bw showed red staining of the face. All other animals were of normal appearance and behaviour. In phase II of the main study, at 1000 mg/kg bw, three females exhibited red-stained face on the day of treatment, and one male exhibited a scab on its face on days 2 through 14. The onset of clinical signs at dose levels of 2000 mg/kg bw and higher was dose related; clinical signs included hypoactivity, staggering gait, hunched posture, prostration, red-stained face, miosis, lacrimation, salivation, tachypnoea, dyspnoea, soft faeces, yellow staining of the urogenital area, tonic or clonic convulsions and tremors. The surviving animals at 2000 and 3000 mg/kg bw returned to a normal appearance by day 3 after treatment.

The acute oral LD_{50} (and 95% confidence limits) were calculated to be 2804 mg/kg bw (1947–4037 mg/kg bw) for males, 2000 mg/kg bw (1354–2954 mg/kg bw) for females and 2450 mg/kg bw (1942–3090 mg/kg bw) for both sexes combined (Glaza, 1997a).

In an acute oral toxicity study conducted according to OECD Test Guideline No. 401, male and female Crl:CD-1[ICR]BR (SPF) mice received dinotefuran (purity 96.5%) in 0.5% aqueous methylcellulose. Initially, a dose range–finding study was performed comprising four groups of one animal of each sex treated at 20 ml/kg bw with a dose level of 500, 1000, 3000 or 5000 mg/kg bw. In the main study, three groups of five animals of each sex were treated at 20 ml/kg bw with 1000, 2000 or 3000 mg/kg bw. The animals were deprived of feed for approximately 5 hours before treatment. Range-finding study animals were checked for mortality once daily for 14 days, and necropsy was not performed. In the main study, animals were checked twice daily for morbidity or mortality. Clinical signs were recorded frequently on the day of treatment and then daily for 14 days. Body weights were recorded predosing and on days 7 and 14 or on the day of death. The animals dying during the

observation period and all survivors were submitted for necropsy and abbreviated postmortem examination.

In the range-finding study, both animals treated at 5000 mg/kg bw and the male treated at 3000 mg/kg bw died on the day of treatment; all other animals survived the observation period (Table 20). In the main study, deaths occurred at dose levels of 2000 mg/kg bw and above, but not at 1000 mg/kg bw. All deaths in the main study occurred on the day of treatment. Transient clinical signs of toxicity, on the day of treatment only, were apparent at dose levels of 2000 mg/kg bw and higher and included hypoactivity, staggering gait, dyspnoea, tonic convulsions and tremors. Survivors treated at 2000 or 3000 mg/kg bw gained weight throughout the observation period. Necropsy and postmortem examination revealed no gross lesions in either decedents or survivors killed at the end of the observation period.

Table 20. Results of the acute oral toxicity study in mice

Dose (mg/kg bw)	No. of animals dying/no. tested			
	Range-finding study		Main study	
	Males	Females	Males	Females
500	0/1	0/1	—	—
1000	0/1	0/1	0/5	0/5
2000	—	—	1/5	2/5
3000	1/1	0/1	4/5	4/5
5000	1/1	1/1	—	—

From Glaza (1997b)

The acute oral LD_{50} (and 95% confidence limits) were calculated to be 2450 mg/kg bw (1801–3331 mg/kg bw) for males, 2275 mg/kg bw (1537–3369 mg/kg bw) for females and 2371 mg/kg bw (1884–2983 mg/kg bw) for both sexes combined (Glaza, 1997b).

In an acute dermal toxicity study conducted according to OECD Test Guideline No. 402, a group of five male and five female Crl:CD[SD]BR (SPF) rats was exposed to dinotefuran (purity 96.5%) at a single dose of 2000 mg/kg bw, formulated as a paste in aqueous carboxymethylcellulose solution, by occluded dermal application for 24 hours to an area of 16 cm^2 of intact clipped dorsal skin. The animals were observed for 14 days and then subjected to necropsy and postmortem examination. All animals survived to the end of the study, and there were no treatment-related clinical signs of toxicity. All male animals gained weight throughout the study, but four females during the 1st week and two females during the 2nd week showed minor weight losses of up to 9 g. Transient slight to moderate erythema, associated with slight oedema in one animal, occurred in 8 of the 10 animals on the day of patch removal. Slight erythema persisted in two animals until day 7, but thereafter no dermal reactions were evident. Necropsy and postmortem examination did not reveal any gross lesions. Based on these results, the acute dermal LD_{50} was estimated to be greater than 2000 mg/kg bw in both sexes (Glaza, 1997c).

In an acute inhalation toxicity study conducted according to OECD Test Guideline No. 403, groups of five male and five female Crl:WI[Glx/BRL/Han]BR (SPF) rats were exposed (nose-only) to a dust aerosol of dinotefuran (purity 93.0%) at a concentration of 0 or 4.09 mg/l for 4 hours. The animals were checked twice daily for morbidity and mortality. The animals were observed for clinical signs hourly during and following exposure and daily thereafter for 14 days. Body weights were recorded before and after exposure on day 1 and on days 2, 8 and 15 of the test. After a 14-day observation period, all animals were submitted for necropsy and a full internal and external

postmortem examination. The nasal cavity and respiratory tract were assessed for evidence of irritation, and the weight of the lungs with trachea was recorded. All gross lesions were preserved in fixative, but not examined histologically. The mean achieved concentration in the exposure chamber of the treated group was 4.09 mg/l (range 2.0–5.0 mg/l), which is considered to be the maximum technically possible; the mass median aerodynamic diameter (MMAD) ± geometric standard deviation (GSD) was 4.74 ± 2.79 µm.

No deaths occurred during the exposure or observation periods, and no treatment-related clinical signs of an adverse reaction to treatment were apparent. Body weight gains were not affected by exposure to dinotefuran. Necropsy and postmortem examination did not reveal any treatment-related lesions in either sex. The group mean absolute and relative lung weights were considered to be unaffected by treatment.

Under the conditions of the study, the acute median lethal concentration (LC_{50}) for male and female rats was estimated to be greater than 4.09 mg/l air (Shepherd, 1999, 2000a,b).

(b) Dermal and ocular irritation and dermal sensitization

The results of the studies on acute dermal and eye irritation and skin sensitization are summarized in Table 21.

Table 21. Summary of acute dermal and eye irritation and skin sensitization studies with dinotefuran

Species	Strain	Sex	Endpoint	Purity (%)	Result	Reference
Rabbit	New Zealand White	M+F	Skin irritation	96.5	Not irritating	Glaza (1998a)
Rabbit	New Zealand White	M+F	Eye irritation	96.5	Slightly irritating	Glaza (1998b)
Rabbit	New Zealand White	M+F	Eye irritation	98.9	Minimally irritating	Kuhn (2004)
Guinea-pig	Crl:[HA]BR	M	Skin sensitization (M&K test)	96.5	Not sensitizing	Glaza (1997d)

F, female; M, male; M&K, Magnusson and Kligman

In an acute dermal irritation study conducted according to OECD Test Guideline No. 404, 0.5 g of dinotefuran (purity 96.5%) moistened with distilled water was applied once for 4 hours, under semi-occlusive dressing, to the shaved intact dorsal and/or flank skin (2.5 × 2.5 cm) of six (five male, one female) New Zealand White (Hra:(NZW)SPF) rabbits. After 4 hours, the occlusive dressings were removed and the test sites washed with water to remove all traces of test article. Skin reactions were evaluated 30 minutes after patch removal and subsequently at 24, 48 and 72 hours. The untreated skin of each animal served as the reference site for grading. Dermal erythema, eschar formation and oedema were graded according to the Draize scoring method, and the scores were calculated for each animal.

Three of the six test animals showed very slight (grade 1) erythema reactions 30 minutes after patch removal. Very slight erythema persisted in one of these animals until 24 hours, but was not apparent in the other two animals at this time. The remaining three animals showed no evidence of dermal irritation at any of the observation intervals. Oedema and other manifestations of dermal irritation did not occur in any animal at any observation interval. According to the EU and Globally Harmonized System of Classification and Labelling of Chemicals (GHS) classification system, five of the six animals had index scores of 0.0, and the remaining animal had an index score of 0.33. Consequently, dinotefuran is not classified as irritating to skin according to EU and GHS criteria. According to the USEPA classification system, the primary dermal irritation index for dinotefuran was calculated as 0.2, a score defined as indicating slight irritation (category IV) (Glaza, 1998a).

In an acute eye irritation study conducted according to OECD Test Guideline No. 405, 0.1 g of dinotefuran powder (purity 96.5%) was introduced into the right conjunctival sac of nine (six male and three female) New Zealand White (Hra:(NZW)SPF) rabbits. The left eye remained untreated as a reference control. Both eyes of three male animals were flushed with water for 1 minute starting 30 seconds after instillation of the test article. The eyes of the other six animals remained unwashed. The treated eyes of all animals were examined for ocular irritation reactions 1, 24, 48, 72 and 96 hours and 7 and 14 days after instillation. Irritation reactions were graded and scored according to the Draize technique. Sodium fluorescein examinations were performed to assist the visualization of possible corneal lesions at 24, 48, 72 and 96 hours or until a negative response was evident.

The unwashed eyes of four of six animals showed slight (grade 1) corneal opacity with epithelial peeling and iridial irritation in two of six animals within 24 hours of instillation of dinotefuran. The reaction was accompanied by slight to moderate (grade 1 or 2) erythema, chemosis and clear or purulent discharge in all six animals. The eyes of four of the animals had returned to normal in appearance within 96 hours of instillation, and the remaining two animals had returned to normal 14 days after instillation (Tables 22 and 23).

Table 22. Summary of positive ocular reactions[a] in the acute eye irritation study in rabbits

Observation	1 h	24 h	48 h	72 h	96 h	7 days	14 days
Unwashed group							
Corneal opacity	1/6	4/6	1/6	1/6	1/6	1/6	0/6
Iris lesion	1/6	2/6	1/6	0/6	0/6	0/6	0/6
Conjunctival redness	6/6	6/6	2/6	1/6	0/6	1/6	0/6
Chemosis	6/6	2/6	1/6	0/6	0/6	0/6	0/6
Washed group							
Corneal opacity	0/3	3/3	1/3	0/3	0/3	0/3	0/3
Iris lesion	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Conjunctival redness	3/3	3/3	2/3	1/3	1/3	0/3	0/3
Chemosis	3/3	2/3	1/3	1/3	1/3	0/3	0/3

From Glaza (1998b)

[a] A positive reaction for each parameter is defined as any corneal opacity or iris lesion score of 1 or greater or any conjunctival redness or chemosis score of 2 or greater.

Table 23. Mean scores (according to EU criteria) in the acute eye irritation study in rabbits

Observation	Unwashed group					Washed group				
	1 h	24 h	48 h	72 h	96 h	1 h	24 h	48 h	72 h	96 h
Corneal opacity	0.2	0.7	0.2	0.2	0.2	0.0	1.0	0.0	0.0	0.0
Iris lesion	0.2	0.3	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Erythema	2.0	2.0	1.3	0.8	0.3	2.0	2.0	1.7	1.3	1.0
Oedema	2.0	1.5	1.2	0.3	0.2	2.3	1.7	1.3	0.7	0.7

From Glaza (1998b)

A positive reaction occurred in three of three animals in the washed group and was characterized by slight corneal opacity and slight to moderate erythema, chemosis and discharge. With the exception of one of three animals that showed slight erythema and chemosis on day 7, the washed eyes of all animals returned to normal in appearance by 96 hours. The appearance of the eyes of the third animal had returned to normal by day 14 (Tables 22 and 23).

Based on these results, dinotefuran is slightly irritating to the eyes of the rabbit but does not require classification according to EU and GHS criteria (Glaza, 1998b).

In an acute eye irritation study conducted according to USEPA guideline OPPTS 870.2400, 0.1 ml (37.8 mg) of dinotefuran powder (purity 98.9%) was introduced into the right conjunctival sac of three (one male and two female) New Zealand White rabbits. The left eye remained untreated to serve as a reference control. All treated eyes were washed with deionized water at room temperature for 1 minute immediately after recording the 24-hour observation. The treated eyes of all animals were examined for ocular irritation reactions 1, 24, 48 and 96 hours after instillation according to the Draize technique. Fluorescein dye retention was assessed for the corneas at 24 hours. One hour after test substance instillation, two animals exhibited minimal corneal effects (slight dulling of normal luster), all three animals had slight conjunctival discharge (grade 1) and one animal exhibited slight conjunctival redness (grade 1). Based on the irritation scoring method of Kay & Calandra (1962), a maximum average irritation score of 2.7 was obtained at 1 hour after treatment. All animals were free of eye irritation at 24 hours after instillation. Fluorescein staining did not occur in any of the eyes. Based on these results, dinotefuran is considered to be minimally irritating to the eyes of the rabbit but does not require classification according to EU and GHS criteria (Kuhn, 2004).

The skin sensitization potential of dinotefuran (purity 96.5%) was investigated in male albino (Crl:[HA]BR) guinea-pigs using a Magnusson and Kligman maximization test according to OECD Test Guideline No. 406. The concentrations of dinotefuran used in the main study were determined in a preliminary irritation study in which two groups of four guinea-pigs were exposed to dinotefuran by occluded topical application for 24 hours at concentrations of 5%, 10%, 15% and 25% weight per weight (w/w) in petrolatum (with each animal receiving two different concentrations) or intradermally at concentrations of 1%, 5%, 10% and 15% weight per volume (w/v) in 0.5% aqueous carboxymethylcellulose (with each animal receiving all four concentrations of the test material). Dermal reactions were evaluated 24 and 48 hours after treatment.

No dermal irritation occurred at any concentration of dinotefuran administered by topical application, up to 25% w/w in petrolatum. In the four animals treated by intradermal injection, dinotefuran produced mild erythema (grade 1) at 1% w/v, mild to moderate diffuse erythema (grades 1–2) reactions at 5% w/v and moderate to marked erythema (grades 2–3) at 10% and 15% w/v.

In the main study, a test group and a negative control group of 20 male guinea-pigs each were used. On day 1, the test group received three pairs of intradermal injections of 50% Freund's complete adjuvant in water, 5% w/v dinotefuran in aqueous carboxymethylcellulose and 10% w/v dinotefuran in aqueous carboxymethylcellulose diluted 1:1 with Freund's complete adjuvant. The control group received similar injections, but without dinotefuran. On day 7, the application sites of both groups of animals were treated topically with 10% sodium lauryl sulfate. On day 8, the animals were treated topically, over the injection sites, under occlusive dressing for 48 hours with 25% w/w dinotefuran in petrolatum (treated group) or petrolatum alone (irritation control group). Two weeks later, both groups were challenged topically, under occlusive dressing for 24 hours, with 25% w/w dinotefuran in petrolatum (right side) and petrolatum alone (left side). The challenge sites were scored for dermal reactions 24 and 48 hours after removal of the challenge dressings. Clinical observations were recorded daily, and individual body weights were recorded pretest and at termination.

There were no treatment-related clinical signs or adverse effects on body weight. None of the test or control group animals exhibited a dermal response to the challenge application of the test or control articles either 24 or 48 hours after patch removal. Therefore, all dermal reaction scores were zero.

It was concluded that dinotefuran does not have a sensitizing effect on the skin of the guinea-pig in the maximization test under the test conditions chosen (Glaza, 1997d).

2.2 *Short-term studies of toxicity*

(a) *Oral administration*

Mice

In a study of toxicity conducted according to OECD Test Guideline No. 407, groups of 10 male and 10 female Crl:CD-1®(ICR)BR VAF/Plus® mice were fed diets containing dinotefuran (purity 96.5%) at a concentration of 0, 5000, 25 000 or 50 000 ppm (equal to 0, 901, 4612 and 10 303 mg/kg bw per day for males and 0, 1043, 5359 and 12 289 mg/kg bw per day for females, respectively) for at least 4 weeks. Morbidity/mortality checks were performed twice daily, and a detailed clinical examination was performed weekly. Body weights and feed consumption were recorded weekly throughout the study. Haematology and serum clinical chemistry analyses were performed in week 5, each on five mice of each sex per group, which had feed withdrawn for at least 4 hours prior to blood sampling. Decedents and all surviving animals killed at the end of the study were subjected to necropsy and postmortem examination of major organs and tissues. Selected organs were weighed, and all preserved tissues from animals treated at 0 or 50 000 ppm were examined by light microscopy. Gross lesions were also examined from all animals treated at 5000 and 25 000 ppm.

There were no treatment-related deaths or clinical signs at any dose level. Both sexes treated at 25 000 or 50 000 ppm showed a dose-related depression of overall body weight gain of between 46.2% and 85.5% (Table 24). Males and females treated at 50 000 ppm lost weight during the 1st week of treatment but subsequently gained weight at a comparable rate to the controls. Marked feed spillage by the groups treated at 25 000 or 50 000 ppm suggested that these test diets were less palatable than untreated diet and precluded a valid assessment of feed consumption for most time points. Therefore, initial weight loss and/or overall depressed weight gain at 25 000 and 50 000 ppm might be a reflection of reduced palatability of the diets. At 5000 ppm, there was no evidence of an effect on feed consumption.

Table 24. Summary of selected findings in the 4-week mouse study

	Males				Females			
	0 ppm	5000 ppm	25 000 ppm	50 000 ppm	0 ppm	5000 ppm	25 000 ppm	50 000 ppm
Body weight (g), week 1	30.7	32.1	32.6	32.8	25.5	26.1	26.5	26.0
Body weight (g), week 5	36.2	37.0	35.4	33.6	29.4	30.4	28.6	27.8
Body weight gain (g), weeks 1–5	5.5	4.9	2.8*	0.3*	3.9	4.3	2.0*	1.8*
Serum total protein (g/dl)	4.7	4.8	5.0	5.1*	4.8	5.0	5.1	4.9
Serum albumin (g/dl)	3.2	3.2	3.3	3.6*	3.5	3.4	3.7	3.5

From Weiler (1997b)
* $P < 0.05$

There were no treatment-related effects on the haematological profile at any dose level. Treatment-related effects on serum chemistry were confined to the male group treated at 50 000 ppm and comprised slightly higher serum total protein and albumin concentrations (Table 24). These minor differences from the controls were not associated with overt histopathological changes and are considered not to be adverse effects. All other serum chemistry values were unaffected by treatment at all dose levels.

There were no treatment-related effects on absolute or relative organ weights and no treatment-related macroscopic or microscopic histopathological alterations at any of the dose levels employed.

The no-observed-adverse-effect level (NOAEL) was 5000 ppm (equal to 901 mg/kg bw per day in males and 1043 mg/kg bw per day in females), based on reduced body weight gain in both sexes at 25 000 ppm and above (Weiler, 1997b).

In a study of toxicity conducted according to OECD Test Guideline No. 408, groups of 10 male and 10 female Crl:CD-1®(ICR)BR VAF/Plus® mice were fed diets containing dinotefuran (purity 96.5%) at concentrations of 0, 500, 5000, 25 000 or 50 000 ppm (equal to 0, 81, 844, 4442 and 10 635 mg/kg bw per day for males and 0, 102, 1064, 5414 and 11 560 mg/kg bw per day for females, respectively) for at least 13 weeks. Morbidity/mortality checks were performed twice daily, and a detailed clinical examination was performed weekly. Body weights and feed consumption were recorded weekly throughout the study. Ophthalmic examinations were conducted pretest and in week 14 on all animals. Haematology, serum clinical chemistry and urine analysis were performed in week 14 on five mice of each sex per group (haematology and clinical chemistry) and on all animals for urine analysis. Blood samples were withdrawn after a period of at least 4 hours of feed deprivation. All animals were subjected to necropsy and postmortem examination of major organs and tissues. Selected organs were weighed, and all preserved tissues from animals treated at 0 or 50 000 ppm were examined by light microscopy. Gross lesions, kidneys, lungs and liver with gall bladder were also examined from all animals treated at 500, 5000 or 25 000 ppm.

There were no deaths and no treatment-related clinical signs at any dose level, but both sexes at 50 000 ppm lost weight during the 1st week of treatment and subsequently showed a treatment-related depression of body weight gain. The overall mean weight gains and body weights at termination of both sexes were reduced. The overall weight gains of males at 25 000 ppm and females at 500, 5000 and 25 000 ppm were 15.9–31.4% lower than, but not significantly different from, those of the controls (Table 25). Increased feed spillage occurred during the 1st week in the groups treated at 25 000 or 50 000 ppm and continued throughout the study at 50 000 ppm. Spillage at concentrations of 25 000 ppm and higher is considered to indicate reduced diet palatability, and any apparent increase in feed consumption at these concentrations is considered to represent spillage. There was no evidence of an effect on feed consumption or feed efficiency at dietary concentrations up to 5000 ppm.

There were no treatment-related ophthalmological or haematological effects at any dose level. Treatment-related effects on serum chemistry after 13 weeks of treatment were confined to males treated at 50 000 ppm, which showed slightly raised serum albumin concentration. Urinary pH was slightly lower in both sexes at 50 000 ppm. These minor differences from the controls were not associated with overt histopathological alterations and are considered not to be adverse effects.

There were no treatment-related effects on absolute or relative organ weights at any dose level, with the exception of lower absolute weights of the heart and liver in females and of the kidneys in both sexes at 50 000 ppm. The differences are considered to be a consequence of growth retardation, as the organ to body weight ratios were not affected. There were no treatment-related macroscopic findings at necropsy at any dose level. There were no treatment-related histopathological alterations, and the nature, severity and incidence of microscopic findings were similar in all treated and control groups.

The NOAEL was 25 000 ppm (equal to 4442 mg/kg bw per day for males and 5414 mg/kg bw per day for females), based on reduced body weight and body weight gain in both sexes at 50 000 ppm (equal to 10 635 mg/kg bw per day for males and 11 560 mg/kg bw per day for females) (Weiler, 1997d, 2000b).

Table 25. Summary of selected findings in the 13-week mouse study

	Males					Females				
	0 ppm	500 ppm	5000 ppm	25 000 ppm	50 000 ppm	0 ppm	500 ppm	5000 ppm	25 000 ppm	50 000 ppm
Body weight (g)										
- week 1	31.0	31.6	30.2	30.1	31.1	26.0	26.1	25.3	26.1	25.6
- week 2	32.7	33.2	32.0	31.2	30.2	27.3	27.2	26.5	26.8	24.0
- week 3	34.4	35.0	33.7	32.9	30.4*	28.9	28.5	27.6	28.0	24.7*
- week 4	34.6	34.9	34.2	32.8	30.9*	29.1	28.5	27.8	28.1	25.1*
- week 8	37.9	38.1	37.0	36.1	33.2*	31.5	30.7	30.0	30.2	26.6*
- week 14	41.7	41.9	40.3	39.1	35.3*	36.5	34.1	32.5*	33.3	28.5*
Body weight gain (g), weeks 1–14	10.7	10.3	10.1	9.0	4.2*	10.5	8.0	7.2	7.2	2.9*
Feed consumption (g)										
- weeks 1–4	45.1	43.6	43.6	43.7	49.6	41.4	43.2	43.6	44.5	46.1
- weeks 5–8	45.6	44.4	45.1	45.8	51.4	45.0	46.1	45.1	45.9	42.3
- weeks 9–13	40.5	40.4	40.2	41.7	45.3	42.6	42.1	42.6	43.7	41.9
Serum albumin (g/dl)	2.9	3.1	3.2	3.3	3.4*	3.4	3.4	3.4	3.5	3.6
Urinary pH	7.3	7.5	7.5	6.9	6.3	7.2	7.2	7.2	6.8	6.6*

From Weiler (1997d)
* $P < 0.05$

Rats

In a study of toxicity conducted according to OECD Test Guideline No. 407, groups of five male and five female Crl:CD®(SD)BR VAF/Plus® rats were fed diets containing dinotefuran (purity 96.5%) at a concentration of 0, 5000, 25 000 or 50 000 ppm (equal to 0, 390, 1814 and 3720 mg/kg bw per day for males and 0, 450, 2183 and 4222 mg/kg bw per day for females, respectively) for at least 4 weeks. Morbidity/mortality checks were performed twice daily, and a detailed clinical examination was performed weekly. Body weights and feed consumption were recorded weekly. Haematology, serum clinical chemistry and urine analyses were performed in week 5. All animals were subjected to necropsy and postmortem examination of major organs and tissues. Selected organs were weighed, and all major organs and tissues from animals treated at 0 or 50 000 ppm were examined by light microscopy. Gross lesions were also examined from animals in the groups treated at 5000 or 25 000 ppm.

There were no deaths and no treatment-related clinical signs at any dose level. Overall reduced weight gain occurred at 25 000 and 50 000 ppm (Table 26). Feed consumption of both sexes at 50 000 ppm and of males at 25 000 ppm was significantly lower during the 1st week of the study, but the differences lessened by the 3rd and 4th weeks. The lower feed consumption data suggest that the diets at 25 000 and/or 50 000 ppm were less palatable, but were eventually accepted by the animals. There were no treatment-related effects on weight gain or feed consumption at 5000 ppm.

Table 26. Summary of selected findings in the 4-week rat study

	Males				Females			
	0 ppm	5000 ppm	25 000 ppm	50 000 ppm	0 ppm	5000 ppm	25 000 ppm	50 000 ppm
Body weight (g)								
- week 1	256	257	264	262	152	161	158	155
- week 5	433	430	403	356*	219	215	208	189
Body weight gain (g), weeks 1–5	177	173	139*	94*	67	53	50	34*
Feed consumption (g)								
- week 1	196	184	161*	115*	115	121	103	80*
- week 2	207	207	186	159*	116	118	113	105
- week 3	205	204	188	179	117	113	110	109
- week 4	164	176	157	184	129	127	117	102
Serum glucose (mg/dl)	118	119	111	101*	105	105	103	100
Serum cholesterol (mg/dl)	56	52	70*	80*	85	70	83	86

From Weiler (1997a)
* $P < 0.05$

There were no treatment-related effects on haematology or urine analysis parameters at any dose level. Treatment-related effects on serum clinical chemistry were confined to the male groups treated at 25 000 and 50 000 ppm: glucose concentration at 50 000 ppm was slightly reduced, and cholesterol concentrations at 25 000 and 50 000 ppm were increased relative to the controls. These differences from the controls were not apparent in the females at any dose level and were not associated with overt histopathological alterations at 50 000 ppm.

There were no effects on organ weights or weight ratios considered to be directly related to treatment with dinotefuran. A number of absolute organ weights were slightly, but significantly ($P < 0.05$), lower in males at 50 000 ppm (spleen, heart, kidneys, liver) and females at 50 000 ppm (heart and ovaries). However, these differences were not apparent in the corresponding body weight ratios. Therefore, the differences in absolute organ weights are considered to be a consequence of the lower terminal body weights. There were no treatment-related macroscopic lesions at any dose level and no treatment-related microscopic findings at 50 000 ppm.

The NOAEL in males was 5000 ppm (equal to 390 mg/kg bw per day), based on reduced body weight gain and increased serum cholesterol at 25 000 ppm (equal to 1914 mg/kg bw per day) and above. The NOAEL in females was 25 000 ppm (equal to 2183 mg/kg bw per day), based on reduced body weight gain at 50 000 ppm (equal to 4222 mg/kg bw per day) (Weiler, 1997a).

In a study of toxicity conducted according to OECD Test Guideline No. 408, groups of 10 male and 10 female Crl:CD®(SD)BR VAF/Plus® rats were fed diets containing dinotefuran (purity 96.5%) at a concentration of 0, 500, 5000, 25 000 or 50 000 ppm (equal to 0, 34, 336, 1623 and 3156 mg/kg bw per day for males and 0, 38, 384, 1871 and 3616 mg/kg bw per day for females, respectively) for at least 13 weeks. Morbidity/mortality checks were performed twice daily, and a detailed clinical examination was performed weekly. Body weights and feed consumption were recorded weekly throughout the study. Ophthalmic examinations were conducted pretest and in week 14 on all animals. Haematology, serum clinical chemistry and urine analysis were performed on all animals pretest and in week 14, following overnight feed deprivation. All animals were subjected to necropsy and postmortem examination of major organs and tissues. Selected organs were weighed, and samples of all major organs and tissues were preserved. All preserved tissues from animals treated at 0 or 50 000 ppm were examined by light microscopy. Gross lesions, adrenal glands,

kidneys, liver and lungs were also examined from all animals in the groups treated at 500, 5000 and 25 000 ppm.

There were no deaths and no treatment-related clinical signs during the study. Males and females treated at 50 000 ppm lost weight during week 1, but subsequently gained weight. The animals of both sexes treated at 25 000 or 50 000 ppm and females treated at 5000 ppm showed statistically significant and dose-related reductions in overall body weight gain (Table 27). At termination, the mean body weights of these groups were 7.2–24.1% lower than control values. The mean weekly feed consumption of both sexes at 25 000 or 50 000 ppm was significantly reduced for at least 11 weeks of the treatment period, and the overall mean feed consumption was 11.5–24.5% lower than control values.

There were no treatment-related ocular lesions at any dose level. Treatment-related effects on haematology and serum clinical chemistry after 13 weeks of treatment were confined to the group treated at 50 000 ppm. Activated partial thromboplastin times (APTT) were slightly shorter than control values and urea nitrogen concentrations were slightly elevated in both sexes, but were significantly different from the controls in males only. Males at 50 000 ppm also showed slightly, but significantly, lower blood glucose, total protein and globulin concentrations. These minor differences from the controls were not associated with overt histopathological alterations and are considered not to be of toxicological significance. All other haematological and serum clinical chemistry parameters were comparable to control group values. Urine analysis profiles were unaffected by treatment at all dose levels.

There were no treatment-related macroscopic findings at necropsy. There were no effects on absolute organ weights or ratios that are considered to be a direct effect of treatment at any dose level. However, absolute heart, kidney, liver and spleen weights were significantly ($P < 0.05$) lower than those of the controls and their ratios relative to body weight and/or brain weight were significantly different from the controls in the groups treated at 25 000 and 50 000 ppm. The absolute weights of the adrenals and pituitary were lower in females and the relative weights of brain and testes were higher in animals at 50 000 ppm. As the body weights of these groups were significantly reduced at termination and because there were no correlating histopathological changes in these organs, the differences from control values are considered to be incidental to treatment or secondary to substantially reduced weight gain.

Treatment-related histopathological alterations were confined to increased cytoplasmic vacuolation of the adrenal cortex in both sexes treated at 25 000 and 50 000 ppm and in males at 5000 ppm (Table 27). The vacuolation was apparent in both the zona glomerulosa and zona fasciculata of the males, but was confined to the zona glomerulosa in the females. The severity of the lesion was graded as minimal or slight in all instances except for one female at 50 000 ppm, which was graded moderate. The nature, incidence and severity of all other histopathological findings in animals treated at 0 or 50 000 ppm did not indicate an effect of treatment with dinotefuran.

The NOAEL was 500 ppm (equal to 38 mg/kg bw per day), based on reduced body weight and body weight gain in females at 5000 ppm (equal to 384 mg/kg bw per day) and above (Weiler, 1997c, 2000a).

Dogs

In a range-finding study of toxicity, not conducted in accordance with GLP, groups of one male and one female Beagle dog were fed diets containing dinotefuran (purity 92.9%) at a concentration of 0, 1250, 5000 or 20 000 ppm for 7 days. After 1 week of treatment, the intermediate- and high-dose groups were killed and subjected to necropsy, and the control and low-dose groups were continued on treatment for a further 7 days at increased dietary concentrations of 40 000 and 30 000 ppm, respectively. The dietary concentration of 40 000 ppm was calculated to provide approximate dose levels of 770 and 924 mg/kg bw per day in males and females, respectively, Morbidity/mortality checks were performed twice daily, and clinical signs of a reaction to treatment were recorded 1 hour before and after treatment. Body weights were recorded before treatment and

feeding on days 0, 2, 4 and 7 and additionally on days 10 and 14 for animals treated for 2 weeks. Feed consumption was recorded daily. Blood and urine were sampled predosing and after 7 and 14 days (40 000 and 30 000 ppm only) of treatment. Urine samples were collected from water-loaded (200 ml) animals and semiquantitatively analysed. Blood samples were withdrawn after overnight feed withdrawal and analysed for haematological and clinical chemistry parameters. All animals were killed after 7 days (1250 and 5000 ppm) or 14 days (0/40 000 and 1250/30 000 ppm) of treatment and subjected to necropsy and postmortem examination. Major organs were weighed, and a comprehensive range of tissues was processed for histological examination. No statistical analyses were performed.

Table 27. Summary of selected findings in the 13-week rat study

	Males					Females				
	0 ppm	500 ppm	5000 ppm	25 000 ppm	50 000 ppm	0 ppm	500 ppm	5000 ppm	25 000 ppm	50 000 ppm
Body weight (g)										
- week 1	257	252	257	258	256	203	195	201	195	195
- week 2	309	305	306	290*	252*	227	222	226	207*	190*
- week 14	572	568	552	515*	446*	345	326	320*	291*	262*
Body weight gain (g), weeks 1–14	315	316	295	257*	190*	141	131	118*	96*	67*
Feed consumption (g)										
- week 1	192	186	188	157*	100*	151	139	140	119*	89*
- week 2	200	193	194	178*	145*	153	147	149	135	117*
- week 4	219	214	205	191*	171*	170	156*	171	142*	147
- week 7	218	217	212	196*	173*	164	158	158	139*	124*
- week 13	197	194	197	171*	155*	152	135*	136*	123*	112*
Adrenal weight, left										
- absolute (mg)	29.4	32.1	27.8	28.8	25.4	40.5	36.2	35.3*	34.3	31.9*
- relative (% of body weight × 1000)	5.6	6.1	5.5	6.1	6.2	12.9	12.2	12.2	13.0	13.3
Adrenal weight, right										
- absolute (mg)	30.1	29.4	27.2	27.4	24.8	38.2	33.3	36.3	33.3	29.8*
- relative (% of body weight × 1000)	5.8	5.5	5.4	5.8	6.0	12.2	11.2	12.5	12.6	12.5
Adrenal cortex (n = 10)										
Zona glomerulosa, increased vacuolation										
- total	0	0	3	2	4	0	0	0	6	10
- minimal	0	0	3	1	3	0	0	0	6	2
- slight	0	0	0	1	1	0	0	0	0	7
- moderate	0	0	0	0	0	0	0	0	0	1
Zona fasciculata, increased vacuolation										
- total	1	0	2	3	5	0	0	0	0	0
- minimal	1	0	1	3	2	0	0	0	0	0
- slight	0	0	1	0	3	0	0	0	0	0

From Weiler (1997c)
* $P < 0.05$

There were no deaths or treatment-related clinical signs during the study. Males and females treated at 40 000 ppm and females treated at 30 000 or 20 000 ppm showed slight weight loss and reduced feed consumption during the week of exposure (Table 28). As reduced feed consumption in the female groups was concentration dependent, the effects might be attributable to reduced palatability of the diet.

Table 28. Summary of selected findings in the range-finding dog study (dietary administration)

	Males				Females			
	0/40 000 ppm	1250/30 000 ppm	5000 ppm	20 000 ppm	0/40 000 ppm	1250/30 000 ppm	5000 ppm	20 000 ppm
Body weight (kg)								
- day 0	9.2	9.0	9.6	9.4	8.8	8.4	9.2	8.6
- day 2	9.2	9.0	9.8	9.4	9.0	8.4	9.4	8.6
- day 4	9.4	9.0	10.0	9.8	9.2	8.4	9.4	8.8
- day 7	9.0	8.8	10.0	9.8	9.0	8.6	9.4	8.4
- day 10	9.4	9.0	—	—	9.0	8.6	—	—
- day 14	8.8	9.0	—	—	8.8	8.2	—	—
Feed consumption (g/day)								
- week 1	400	357	400	400	339	400	400	265
- week 2	181	257	—	—	208	231	—	—

From Teramoto (1998a)

There were no treatment-related effects at any dose level on urine analysis, haematological or clinical chemistry profiles and no treatment-related gross lesions at necropsy or effects on organ weights. There were no treatment-related histopathological alterations in any tissue at any dose level. The nature and incidence of microscopic changes indicated that they were spontaneous in origin and incidental to treatment with dinotefuran.

In conclusion, administration of dinotefuran by the diet resulted in reduced feed consumption at 30 000 ppm and above in males and at 20 000 ppm and above in females and in decreased body weight at 40 000 ppm in the male dog and at 20 000 ppm and above in female dogs (Teramoto, 1998a).

In a second range-finding study of toxicity, not conducted in accordance with GLP, groups of one male and one female Beagle dog were administered dinotefuran (purity 92.9%) by capsule at a dose level of 0, 30, 100 or 300 mg/kg bw per day for 7 days. Morbidity/mortality checks were performed twice daily, and clinical signs of a reaction to treatment were recorded 1 hour before and after treatment. Body weights were recorded before treatment and feeding on days 0, 2, 4 and 7. Feed consumption was recorded daily. Blood and urine were sampled prior to and after 7 days of treatment. Urine samples were collected from water-loaded (200 ml) animals and semiquantitatively analysed. Blood samples were withdrawn after overnight feed withdrawal and analysed for haematological and clinical chemistry parameters. All animals were killed at the end of the treatment period and subjected to necropsy and postmortem examination. Major organs were weighed, and a comprehensive range of tissues was processed for histological examination. No statistical analyses were performed.

There were no deaths during the study; treatment-related clinical signs were confined to diarrhoea and vomiting in both sexes treated at 300 mg/kg bw per day. Feed consumption and body weight gain were unaffected by treatment at all dose levels (Table 29). No biologically relevant changes in urine analysis, haematological and blood biochemistry profiles occurred at any dose level.

Table 29. Summary of selected findings in the range-finding dog study (capsule administration)

	Males				Females			
	0 mg/kg bw per day	30 mg/kg bw per day	100 mg/kg bw per day	300 mg/kg bw per day	0 mg/kg bw per day	30 mg/kg bw per day	100 mg/kg bw per day	300 mg/kg bw per day
Body weight (kg)								
- day 0	9.8	10.0	10.0	10.0	8.2	7.6	9.4	8.4
- day 7	10.4	10.6	10.4	10.4	8.0	7.6	9.6	8.6
Feed consumption (g/day), days 1–6	400	400	400	400	270	256	400	400
Testes weight								
- absolute (g)	6.87	4.33	5.29	3.29	—	—	—	—
- relative (% of body weight × 100)	6.61	4.08	5.09	3.16	—	—	—	—
Prostate weight								
- absolute (g)	3.00	2.05	1.33	1.80	—	—	—	—
- relative (% of body weight × 100)	2.88	1.94	1.28	1.73	—	—	—	—
Uterus weight								
- absolute (g)	—	—	—	—	0.89	0.79	1.24	0.49
- relative (% of body weight × 100)	—	—	—	—	1.12	1.04	1.29	0.57

From Teramoto (1998b)

There were no treatment-related gross lesions or organ weight changes at necropsy in any of the dinotefuran-treated groups. The absolute and relative organ weights of dinotefuran-treated animals were variable, but there were no trends indicative of an effect of treatment. Although the absolute and relative weights of testis, prostate and uterus in dinotefuran-treated animals were generally lower than control values, the differences are considered to reflect differences in the stage of sexual maturity rather than an effect of treatment (Table 29).

There were no treatment-related histopathological alterations in any tissue at any dose level. The nature and incidence of microscopic changes indicated that they were spontaneous in origin and incidental to treatment with dinotefuran.

In conclusion, administration of dinotefuran by capsules resulted in higher incidences of vomiting and diarrhoea at 300 mg/kg bw per day (Teramoto, 1998b).

In a study of toxicity conducted according to OECD Test Guideline No. 409, groups of four male and four female Beagle dogs were fed diets containing dinotefuran (purity 93.0%) at a concentration of 0, 1600, 8000 or 40 000 ppm for 13 weeks. Because of a marked reduction in feed consumption, the highest dietary concentration was reduced to 30 000 ppm on day 5 and further to 24 000 ppm on day 12. Mean achieved dose levels were 0, 58, 307 and 862 mg/kg bw per day for males and 0, 58, 323 and 950 mg/kg bw per day for females, respectively. Morbidity and mortality were checked twice daily, and clinical signs were recorded daily. Feed consumption was measured daily for the first 2 weeks of treatment and weekly thereafter. Water consumption was recorded for 2 days/week. Body weights were recorded weekly and at necropsy. Ophthalmic examinations were conducted pretest and in week 14 on all animals. Haematology, serum clinical chemistry and urine analysis were performed on all animals, after overnight feed withdrawal, pretest and in weeks 5 and 14. All animals were subjected to necropsy and postmortem examination of major organs and tissues. Selected organs were weighed, and samples of all preserved tissues from all animals were examined by light microscopy.

There were no deaths during the study. Treatment-related clinical signs were confined to animals treated at 40 000/30 000 ppm. Discoloured faeces, few or no faeces, thinness, slightly reduced activity and pale gums occurred in some animals. Two males and one female initially treated at 40 000 ppm showed black faeces for 1 or 2 days, but the occurrence may be related to stress resulting from offering unpalatable diet, rather than a primary effect of dinotefuran. One male treated at 40 000/30 000 ppm showing liquid/mucoid faeces was diagnosed with enteritis on day 8 and was taken off dose for 4 days. There were no other clinical signs after reduction of the dietary concentration to 24 000 ppm.

Body weights were significantly reduced in both sexes at 24 000 ppm from week 2, resulting in overall reductions in weight gain of 33.3% and 31.4% in males and females, respectively (Table 30). However, a large proportion of the reduced weight gain was associated with the administration of 40 000/30 000 ppm during the first 12 days of treatment, and subsequent treatment at 24 000 ppm resulted in only marginally lower weight gains. Females at 1600 and 8000 ppm also showed significantly lower body weights during the last 8 weeks of treatment and lower overall weight gains (34.3% and 31.4%, respectively).

Feed consumption was markedly and significantly reduced in both sexes treated at 40 000/30 000 ppm, indicating reduced diet palatability. Although feed consumption increased after the reduction of the dietary concentration to 24 000 ppm, reduced feed consumption persisted in both sexes throughout the study (Table 30).

There was a concomitant decrease in water consumption during the first 11 days of treatment at 40 000/30 000 ppm. Subsequently, the water consumption of the males remained depressed, but water consumption of the females was comparable to, or higher than, that of the control females from day 24. The feed consumption of females treated at 1600 and 8000 ppm was also low in comparison with the feed consumption of the female controls. However, the differences in feed consumption are considered not to be toxicologically relevant because they were not dose related and the predosing feed consumption at 1600 ppm was 16.4% lower than the control consumption. Water consumption at 1600 or 8000 ppm was not affected by treatment for both sexes.

There were no ocular lesions in any animal after 13 weeks of treatment, and there were no treatment-related haematological changes in either sex at any dose level.

Males and females at 24 000 ppm showed slightly but significantly reduced serum alanine aminotransferase (ALT) activity relative to both predosing values and the controls after 5 and 13 weeks of treatment. Urinary pH of males at 24 000 ppm was marginally lower than control values in weeks 5 and 14 (Table 30). There were no other treatment-related changes in the haematology, serum chemistry or urine analysis profiles at any dose level.

All organ weights and weight ratios were unaffected by treatment at all dose levels, and none at 24 000 ppm was significantly different from the control values. There were no treatment-related macroscopic or microscopic histopathological alterations at any of the dose levels employed. Although haemorrhage in the mesenteric and/or mandibular lymph nodes occurred in three of the four males treated at 24 000 ppm, its occurrence is considered incidental to treatment with dinotefuran, as haemorrhage was not evident in other organs, and none of the macroscopic or clinical pathology observations suggested a haemorrhagic condition in these animals. All other histopathological alterations occurred sporadically and without regard to sex or dose level.

The NOAEL in males was 8000 ppm (equal to 307 mg/kg bw per day), based on a reduction in feed and water consumption, body weight and body weight gain at 24 000–40 000 (equal to an average dose of 862 mg/kg bw per day). In females, a NOAEL could not be identified; the lowest-observed-adverse-effect level (LOAEL) was 1600 ppm (equal to 58 mg/kg bw per day), based on a reduction in feed consumption, body weight and body weight gain at all doses administered (Weiler, 1999a,b).

Table 30. Summary of selected findings in the 13-week dog study

	Males				Females			
	0 ppm	1600 ppm	8000 ppm	24 000 ppm	0 ppm	1600 ppm	8000 ppm	24 000 ppm
Body weight (kg)								
- week 1	8.1	8.0	8.2	8.3	7.1	7.0	7.2	7.0
- week 2	8.9	9.1	8.9	7.9*	7.9	7.7	7.8	6.8*
- week 4	9.9	9.9	9.6	9.1*	8.7	8.2*	8.4	7.8*
- week 8	10.8	11.0	10.6	10.0*	9.8	8.8*	9.0*	8.6*
- week 13	11.7	11.8	11.5	10.7*	10.6	9.3*	9.6*	9.4*
Body weight gain (kg), weeks 1–14	3.5	3.6	3.3	2.5*	3.5	2.3*	2.5	2.3*
Feed consumption (g/week)								
- week 1	2951	2913	2555	1394*	2712	2406	2588	869*
- week 2	2919	2671	2603	2381	2863	2311*	2557	2240*
- week 4	2875	2645	2686	2570	2773	2212*	2392*	2295*
- week 8	2958	2528	2708	2391*	2722	2203*	2350	2207*
- week 13	2990	2722	2896	2411*	2657	2102*	2631	2402
Water consumption (g/week)								
- day 2	915	780	743	138*	573	503	390	38*
- day 10	1455	958*	1260	798*	1030	903	818	748
- day 24	1423	1090	1295	1165	945	790	735	1035
- day 52	1550	980*	1270	963*	977	658	683	940
- day 86	1505	1095	1313	1000*	980	833	747	1103
Urinary pH, mean (range)								
- week −1	7.0 (7.0–7.0)	6.9 (6.5–7.5)	6.9 (6.5–7.0)	7.0 (6.5–7.5)	6.8 (6.5–7.5)	7.1 (6.0–7.5)	7.1 (7.0–7.5)	6.6 (6.5–7.0)
- week 5	8.1 (7.5–8.5)	7.5 (6.5–8.5)	6.8 (6.0–7.5)	6.5 (6.0–7.0)	6.9 (6.5–7.0)	6.8 (6.5–7.0)	6.5 (6.0–7.0)	6.5 (6.0–7.0)
- week 14	7.6 (7.0–8.5)	7.6 (7.5–8.0)	7.8 (7.0–8.5)	6.8 (6.0–7.5)	6.6 (6.5–7.0)	7.3 (6.5–8.0)	7.1 (7.0–7.5)	6.6 (6.0–7.0)
Lymph nodes, mesenteric; haemorrhage	0	0	0	3	1	0	1	2
Lymph nodes, mandibular; haemorrhage	0	0	0	1	0	1	0	0

From Weiler (1999a)
* $P < 0.05$

In a study of toxicity conducted according to OECD Test Guideline No. 452, groups of four male and four female Beagle dogs were fed diets containing dinotefuran (purity 93.0%) at a concentration of 0, 640, 3200 or 16 000 ppm (equal to 0, 20, 111 and 559 mg/kg bw per day for males and 0, 22, 108 and 512 mg/kg bw per day for females, respectively) for 52 weeks. The animals were observed twice daily for morbidity or mortality and daily for clinical signs of poor health or abnormal behaviour. Body weights and feed and water consumption were recorded weekly for 16 weeks and at 4-week intervals thereafter. Ophthalmoscopic examinations were conducted on all animals pretest and during week 52. Haematology, serum clinical chemistry and urine analysis were performed on all

animals pretest and in weeks 14, 27 and 53. Feed was withdrawn from the animals overnight prior to and during sampling. A sample of plasma was retained at each blood sampling interval from each animal and stored deep-frozen for possible future analysis. The animals were killed after at least 52 weeks of treatment and subjected to necropsy and postmortem examination. Feed was withdrawn from the animals overnight prior to necropsy. Organ weights were recorded, and samples of organs and tissues were preserved and prepared for histological evaluation and then examined microscopically from all animals.

All animals survived to the end of the treatment period, and no treatment-related clinical signs of toxicity or ocular defects occurred at any dose level. There was a treatment-related decrease in the overall body weight gains of both sexes at 16 000 ppm and of females at 3200 ppm. Body weight gains were 30–37% lower than the control values and statistically significant in the female groups. The body weight gains of males at 3200 ppm and both sexes at 640 ppm were unaffected by treatment (Table 31). Females treated at 3200 and 16 000 ppm showed 8% and 12% decreases, respectively, in feed consumption, but consumption was unaffected by treatment in the other groups. Water consumption was unaffected by treatment at all dose levels.

Table 31. Summary of selected findings in the 52-week dog study

	Males				Females			
	0 ppm	640 ppm	3200 ppm	16 000 ppm	0 ppm	640 ppm	3200 ppm	16 000 ppm
Body weight (kg)								
- week 1	8.7	8.7	8.9	8.5	8.0	7.9	7.9	7.9
- week 14	11.4	11.4	11.0	11.0	10.8	10.2	9.9*	9.7*
- week 28	11.6	11.7	11.5	10.7	11.3	10.6	10.3	10.2
- week 40	11.7	11.9	12.1	11.1	12.0	11.1	10.4*	10.2*
- week 52	11.7	11.6	11.9	10.6	11.5	11.4	10.1*	10.2*
Body weight gain (kg), weeks 1–52	3.0	2.9	3.0	2.1	3.5	3.6	2.3*	2.3*
Feed consumption (g/day), weeks 1–52	349	330	368	363	350	348	322	307
Thymus weight								
- absolute (g)	7.23	4.88	3.58	3.55	5.61	6.78	3.89	3.88
- relative (% of body weight × 100)	6.16	4.12	3.09	3.36	4.83	5.96	3.93	4.00
Thymus microscopic findings								
- cyst, ultimobranchial	0/4	0/3	3/4	2/4	1/4	1/4	1/4	1/4
- depletion, lymphocytic	0/4	1/3	2/4	0/4	1/4	1/4	1/4	0/4

From Weiler (1999c)
* $P < 0.05$

There were no treatment-related effects at any dose level on the haematological, serum clinical chemistry or urine analysis profiles. The only statistically significant differences between the 16 000 ppm group and the controls were higher urinary pH at week 27 and slightly higher serum albumin and potassium ion concentrations at week 53 in females. These differences are considered incidental to treatment with dinotefuran because they were not evident at other sampling intervals and were not associated with correlative clinical or histopathological alterations. There were no treatment-related effects at any dose level on the incidence of macroscopic findings at necropsy or on organ weights or ratios. An apparent treatment-related effect on the group mean thymus weight of all male treated groups was noted (Table 31). Statistically significant higher uterus to body weight ratios in

females treated at 3200 ppm and higher ovary to body weight ratios in females at 3200 and 16 000 ppm were attributed to lower body weights at termination. There were no treatment-related histopathological alterations at any dose level. All microscopic findings were typical of those occurring spontaneously in Beagle dogs.

A detailed examination of the individual thymus weights in male dogs showed that there was a single high value in one control animal (11.71 g), which represents the highest thymus weight recorded in 18-month-old control dogs at the study performing laboratory (Table 32). This value increases the contemporary control mean thymus weight to an unrepresentative value of 7.23 g (cf. historical control mean value of 5.94 g). Excluding this weight reduces the contemporary control mean to a value of 5.74 g, which is very close to the historical control mean value of 5.94 g. Furthermore, examination of the individual thymus weights showed that there are two dogs, one low-dose and one high-dose animal, with thymus weights (2.00 and 2.09 g, respectively) outside the historical control range (minimum value = 2.33 g). The low-dose male dog, with a thymus weight of 2.00 g, showed minimal lymphocytic depletion in the organ, an alteration that was not apparent in any of the high-dose males. This animal gained only 0.3 kg in weight during the entire 52-week treatment period, in contrast to all other treated males, which gained 1.4–4.4 kg, and to control males, which gained 2.3–3.9 kg. Although the animal gained 2.0 kg up to week 24, thereafter it progressively lost 1.7 kg in weight. As none of the other mid- or high-dose male animals showed a similar pattern of weight loss during the second half of the treatment period, the weight loss in this low-dose animal does not appear to be an effect of treatment, but the reason for it is unclear. Minimal kidney mineralization was the only other notable histomorphological change in this animal. Whatever the cause of weight loss during the latter part of the study was, this animal cannot be regarded as representative of the low-dose group. Therefore, excluding its unusually low thymus weight from the calculation of the group mean value, the low-dose group mean thymus weight is 5.83 g (cf. historical control mean value of 5.94 g and contemporary control mean [excluding aberrant value] of 5.74 g).

Table 32. Mean and individual thymus weights of males in the 52-week dog study[a]

	0 ppm	640 ppm	3200 ppm	16 000 ppm
Thymus weight (g), individual values	6.17	4.19	4.25	4.74
	11.71#	8.08	3.09	3.61
	5.67	5.23	4.37	3.74
	5.37	2.00#	2.60	2.09
Thymus weight (g), mean value	7.23	4.88	3.58	3.55
(mean, excluding one high/low value)	(5.74)	(5.83)		
Thymus weight (% of body weight × 100), individual values	5.5	3.4	3.4	4.6
	9.3#	6.6	2.6	3.3
	4.9	4.5	4.0	3.6
	4.9	2.0#	2.4	2.0
Thymus weight (g), mean value	6.2	4.1	3.1	3.4
(mean, excluding one high/low value)	(5.1)	(4.8)		

From Weiler (1999c); Gale (2005)

Value excluded for calculation of mean value

[a] Historical control data for thymus weights, mean [range]:

- Absolute weight (g): Males (*n* = 39): 5.94 [2.33–11.71]; females (*n* = 42): 4.83 [1.21–10.90]
- Relative weight (% of body weight × 100): Males (*n* = 39): 5.15 [2.23–9.42]; females (*n* = 42): 4.85 [1.49–11.93].

For the evaluation of the thymus weights of the male treated groups, the following facts were considered: 1) there was no corresponding effect on thymus weights in the 13-week dog study at comparable or even higher dose levels; 2) for the thymus weights at the middle and high doses, there

was no dose dependency, although dose levels were 5-fold different; and 3) the lower thymus weights were not correlated with plausible histopathological changes, especially with a depletion of lymphocytic tissue. Therefore, the lower group mean thymus weights in male dogs were not considered to be a treatment-related adverse effect.

The NOAEL in males was 3200 ppm (equal to 111 mg/kg bw per day), based on a reduction in body weight gain at 16 000 ppm (equal to 559 mg/kg bw per day). The NOAEL in females was 640 ppm (equal to 22 mg/kg bw per day), based on a reduction in feed consumption, body weight and body weight gain at 3200 ppm (equal to 108 mg/kg bw per day) and above (Weiler, 1999c; Gale 2005).

(b) Dermal application

Rats

In a range-finding dermal toxicity study, groups of five male and five female Crl:CD®(SD)IGS BR rats were treated with dinotefuran (purity 93.0%) as a suspension in 0.5% aqueous carboxymethylcellulose by dermal application at a nominal dose level of 0, 40, 200 or 1000 mg/kg bw per day for 14 days. Applications were made to clipped, intact dorsal skin sites (approximately 10% of body surface area) for 6–7 hours/day under semi-occluded dressings. The dressings were removed each day at the end of the exposure period, and the application sites were wiped with water to remove remaining dinotefuran formulation. The animals were observed twice daily for morbidity/mortality, and detailed clinical examinations outside the home cage were performed on days 1, 8 and 15. Dermal irritation reactions were scored immediately before application on days 1, 3, 5, 7, 9, 11, 13, 14 and 15. Body weights were recorded weekly starting on the 1st day of treatment. All animals were killed on day 15 and subjected to necropsy and postmortem examination of major organs and tissues. Samples of treated and untreated skin and gross lesions were preserved from all animals and, after histological preparation, examined microscopically.

There were no deaths or clinical signs of systemic toxicity at any dose level during the study. Slight to moderate skin atonia occurred on occasional days in all animals of both sexes treated at 1000 mg/kg bw per day and in all females treated at 200 mg/kg bw per day. Two males at 200 mg/kg bw per day, one control female and a female at 40 mg/kg bw per day also showed slight skin atonia. There was no other indication of dermal irritation at any dose level. In the absence of any dermal irritation (such as erythema or oedema) or any pathological changes in the skin, and due to the transient nature of this observation, the skin atonia was not considered adverse.

There were no treatment-related or statistically significant effects on body weight at any dose level. There were no treatment-related macroscopic or histopathological findings at any dose level. All histopathological findings in treated skin samples were considered to be incidental to treatment.

The NOAEL for systemic toxicity was 1000 mg/kg bw per day, the highest dose tested. The NOAEL for local toxicity was 1000 mg/kg bw per day, the highest dose tested (Henwood, 2001a).

In a study of dermal toxicity conducted in compliance with OECD Test Guideline No. 410, groups of 10 male and 10 female Crl:CD®(SD)IGS BR rats were treated with dinotefuran (purity 93.0%) as a suspension in 0.5% aqueous carboxymethylcellulose by dermal application at a nominal dose level of 0, 40, 200 or 1000 mg/kg bw per day for 29 days. Applications were made to clipped, intact dorsal skin sites (approximately 10% of body surface area) for 6–7 hours/day under semi-occluded dressings. The animals were fitted with flexible collars during exposure. The dressings and collars were removed each day at the end of the exposure period, and the application sites were wiped with water to remove remaining dinotefuran formulation. The animals were observed twice daily for morbidity/mortality, and detailed clinical examinations outside the home cage were performed on days 1, 8, 15, 22 and 29. Changes in posture, reactivity to handling, tonic/clonic movements, stereotypical/bizarre behaviour patterns and gait abnormalities were also assessed weekly. On day 24/25, expanded clinical observations were performed on all animals. Motor activity of all animals

was quantitatively assessed on day 24/25. An ophthalmoscopic examination was performed on all animals predosing and on day 26. Dermal irritation reactions were scored immediately before application on days 1, 8, 15, 22 and 29 and on the day of necropsy (day 30). Body weights were recorded predosing and weekly thereafter, starting on the 1st day of treatment. Feed consumption was recorded weekly. Haematology and clinical chemistry analyses were performed on blood samples collected from all animals (feed deprived) on day 30. All survivors were killed on day 30 and subjected to necropsy, postmortem examination of major organs and tissues and organ weight recording. The decedent (see below) was also subjected to necropsy. A full range of tissues was preserved from all animals, and stained sections from the animals treated at 0 or 1000 mg/kg bw per day and the decedent were examined microscopically. Gross lesions from all animals were examined microscopically.

The death of a male animal treated at 40 mg/kg bw per day was considered incidental to treatment. All other animals survived the scheduled treatment period, and there were no clinical signs of systemic toxicity at any dose level. There were no treatment-related effects on the expanded clinical observation parameters evaluated and no statistically significant effects on quantitative motor activity. No ocular lesions were evident in any animal after 25 days of treatment. One male treated at 40 mg/kg bw per day and two females at 1000 mg/kg bw per day showed slight (grade 1) skin atonia at the application site on one or two occasions during the treatment period. There were no other signs of dermal irritation at any dose level. There were no treatment-related effects at any dose level on the feed consumption or body weight gains of either sex. There were no treatment-related effects in either sex at any dose level on the haematological or clinical chemistry parameters examined or on absolute or relative organ weights or macroscopic findings at necropsy. Treatment-related histopathological alterations were confined to an increase in the incidence and severity of acanthosis/hyperkeratosis in the treated skin of females at 1000 mg/kg bw per day (five animals, severity grade 1; three animals, severity grade 2), whereas this finding occurred in two control females (both animals severity grade 1). In males, all control and 1000 mg/kg bw per day animals showed this skin alteration (severity grades 1 and 2).

The NOAEL for systemic toxicity was 1000 mg/kg bw per day, the highest dose tested. The NOAEL for local toxicity in males was 1000 mg/kg bw per day, the highest dose tested. In females, the LOAEL for local toxicity was 1000 mg/kg bw per day, based on an increase in the incidence and severity of acanthosis/hyperkeratosis in the treated skin; a NOAEL was not determined, as lower doses were not evaluated histopathologically (Henwood, 2001b).

(c) Exposure by inhalation

In a study of inhalation toxicity conducted in compliance with OECD Test Guideline No. 412, groups of 10 male and 10 female Crl:WI(GlxBRL/Han)BR rats were exposed for 6 hours/day for 29 or 30 days, by inhalation in nose-only chambers, to an atmosphere of dinotefuran (purity 99.1%) as a dust in air at a nominal concentration of 0 (air only), 2.89, 16.02 or 61.24 mg/l (equivalent to gravimetrically determined concentrations of 0, 0.22, 0.66 and 2.08 mg/l, respectively). The calculated MMAD ± GSD values were 2.03 ± 3.31 µm, 1.80 ± 3.60 µm and 1.55 ± 2.96 µm, respectively. The animals were observed twice daily for morbidity and mortality. All animals were observed daily for clinical signs immediately after the end of exposure. In addition, each animal was given a detailed physical examination at weekly intervals. Body weights were recorded before exposure on day 1, at weekly intervals and at necropsy. Feed consumption was determined weekly. Ophthalmoscopic examinations were performed on all animals pre-exposure and on all animals treated at 0 or 2.08 mg/l during week 4. Haematology, clinical chemistry and urine analysis were performed on all animals during week 4. Blood and urine samples were collected after overnight deprivation of feed and feed and water, respectively. All animals were subjected to necropsy after overnight deprivation of feed, and a full internal and external postmortem examination was performed. Selected organs were weighed. The tissues from the animals treated at 0 or 2.08 mg/l and gross lesions and respiratory tract tissues from all groups were examined microscopically.

There were no deaths or treatment-related clinical signs in any exposure group. Post-exposure clinical observations were predominantly localized thinning hair, attributable to the restraint procedure. The body weight gains of all male treated groups were significantly lower than the control gain during week 1. Thereafter, weight gains were slightly lower than the control values, but not statistically significant at any exposure concentration. The terminal body weights of the male treated groups were 5.2%, 3.8% and 6.4% lower than the control value, whereas the overall body weight gains were 19%, 21% and 25% lower than the control value, at 0.22, 0.66 and 2.08 mg/l, respectively (Table 33). The weight gain of all female treated groups was comparable to the control values throughout the study. The mean weekly feed consumption of all male treated groups in weeks 1 and 2 and in the high-dose group in week 3 was 4.3–10.7% lower than the control consumption. However, the differences were not statistically significant. The feed consumption of all female treated groups was unaffected by exposure to dinotefuran.

Table 33. Summary of selected findings in the 4-week rat inhalation study

	Males				Females			
	0 mg/l	0.22 mg/l	0.66 mg/l	2.08 mg/l	0 mg/l	0.22 mg/l	0.66 mg/l	2.08 mg/l
Body weight (g)								
- week 0	207.0	205.3	210.6	206.3	163.3	166.6	169.0	168.0
- week 4	271.9	257.8	261.7	254.6	189.3	193.7	190.2	197.6
Body weight gain (g)								
- weeks 0–1	26.7	17.1*	16.0**	14.3**	8.2	7.2	7.1	7.0
- weeks 1–4	38.2	35.4	35.2	34.0	17.8	19.8	14.1	22.5
- weeks 0–4	64.8	52.5	51.1*	48.3**	26.0	27.0	21.2	29.5
Food consumption (g)								
- week 1	146.3	137.8	138.9	130.7	109.5	110.1	109.0	109.7
- week 2	155.1	148.4	147.0	140.0	113.5	117.6	114.2	117.9
- week 3	155.3	153.9	151.3	145.7	117.2	122.8	119.2	120.2
- week 4	134.7	137.3	138.9	135.9	110.7	113.1	114.4	119.8
ALT (IU/l)	31	39	38	43**	41	42	38	41

From Shepherd (2002)
ALT, alanine aminotransferase; IU, international units; * $P < 0.05$; ** $P < 0.01$

There were no treatment-related ophthalmological findings at the highest exposure concentration. There were no treatment-related effects on the haematological or plasma and urine clinical chemistry profiles in either sex at any exposure concentration. There was a minimal, but statistically significant, difference from the controls in the ratio of neutrophils to lymphocytes in males exposed to 2.08 mg/l, but this was considered to reflect the biological variation inherent in small groups of animals. The group mean plasma ALT activity of the male group exposed to 2.08 mg/l was significantly higher than that of the male control group by 38.7%, but as the absolute value was within the range of historical control values, and in the absence of correlating histopathological alterations in the liver, it is not considered to be an adverse effect. All other haematological and clinical chemistry values at 2.08 mg/l were comparable to, and not significantly different from, the control values.

There were no treatment-related macroscopic findings or organ weight changes at any exposure concentration. There were no treatment-related histopathological alterations in the tissues of rats of either sex in the group exposed to 2.08 mg/l. Histopathological alterations were infrequent, minor in nature and consistent with the normal pattern of findings in rats of the strain and age used.

The nature and incidence of all microscopic findings were comparable in the treated and control groups.

The no-observed-adverse-effect concentration (NOAEC) in males was 0.22 mg/l, based on a reduction in body weight gain at 0.66 mg/l and above. The NOAEC in females was 2.08 mg/l, the maximum technically achievable aerosol concentration (Shepherd, 2002).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a study of carcinogenicity conducted according to OECD Test Guideline No. 451, groups of 60 male and 60 female Crl:CD-1®(ICR)BR VAF/Plus® mice were given diets containing dinotefuran (purity 93.0%) at a concentration of 0, 25, 250, 2500 or 25 000 ppm (equal to 0, 3, 34, 345 and 3694 mg/kg bw per day for males and 0, 4, 45, 441 and 4728 mg/kg bw per day for females, respectively) for at least 78 weeks. An additional 10 animals of each sex per group, similarly treated, were killed after at least 52 weeks of treatment for interim evaluation. Animals were observed twice daily for morbidity or mortality, and detailed clinical examinations were performed weekly. Body weights and feed consumption were recorded weekly for 13 weeks and monthly thereafter. The water consumption of 12 animals of each sex per group was measured for 2 days/week each week for 13 weeks and monthly thereafter. Haematological investigations were performed in week 53 on 10 animals of each sex per group and in week 79 on all survivors. Ten animals of each sex per group were killed in week 53, and all remaining animals in week 79. All survivors and decedents were subjected to detailed necropsy and postmortem examination, but no organ weights were recorded in decedents. Organ weights were recorded in 10 animals of each sex per group killed after 52 and 78 weeks of treatment. Tissue/organ samples from all animals were preserved for possible light microscopy. Samples of liver, kidney and adrenal gland from two animals of each sex per group were also preserved for possible electron microscopy. All tissues from decedents and animals treated at 0 or 25 000 ppm and gross lesions, lungs, liver and kidneys from all animals were examined by light microscopy.

Survival was unaffected by treatment at all dose levels. Control male survival in week 79 was 73% compared with 72–83% in the dinotefuran-treated groups. Female control survival in week 79 was 76% compared with 67–80% in the dinotefuran-treated groups. There were no treatment-related clinical signs of toxicity, including the incidences of palpable tissue masses. Overall body weight gains of both sexes were significantly reduced by treatment at 25 000 ppm but were unaffected by treatment at lower dose levels. At 25 000 ppm, overall body weight gains were reduced by 17.9% and 25.2% in males and females, respectively, and group mean body weights at week 78 were 95.3% and 90.9% of control values in males and females, respectively (Table 34). Feed consumption and water consumption were unaffected by treatment at all dose levels. Treatment-related haematological effects were confined to slightly lower platelet counts in both sexes after 78 weeks of treatment at 25 000 ppm. The group mean counts were 19.9% and 17.4% lower than control values in males and females, respectively, but were statistically significant in males only. The effect was not apparent at lower dose levels after 78 weeks or in any group after 52 weeks of treatment.

There were no treatment-related macroscopic pathological findings or organ weight changes in either sex at any dose level after 52 and 78 weeks of treatment. The nature and incidence of non-neoplastic histopathological findings were similar in decedent animals and those surviving to termination, and there were no treatment-related differences between the control and treated groups. The nature and incidence of neoplastic changes were not influenced by treatment with dinotefuran at any dose level. There were no statistically significant trends or group differences in the incidences of any common tumours ($P > 0.01$) or rare tumours ($P > 0.05$), and all differences between the groups are considered to be normal biological variation.

Table 34. Summary of selected findings in the 78-week mouse study

	Males					Females				
	0 ppm	25 ppm	250 ppm	2500 ppm	25 000 ppm	0 ppm	25 ppm	250 ppm	2500 ppm	25 000 ppm
Body weight (g)										
- week 1	31.8	31.3	31.6	32.0	31.8	24.6	24.2	24.1	24.5	24.5
- week 13	39.9	39.8	39.9	39.2	37.9*	31.0	31.1	29.7*	30.1	29.3*
- week 50	46.1	46.7	45.9	45.7	41.6*	37.5	37.1	35.6	35.9	33.8*
- week 78	45.0	45.7	45.1	44.5	42.9	38.6	38.9	37.3	38.2	35.1*
Body weight gain (g), weeks 1–78	13.4	14.6	13.5	12.6	11.0*	13.9	14.8	13.4	13.6	10.4*
Platelet count (10^9/l)										
- week 53	1125	1138	1241	1329	1147	1028	1139	988	1063	1035
- week 79	1405	1309	1545	1245	1125*	1038	1011	1021	966	857
Spleen weight										
- absolute (g)	0.287	0.168	0.129	0.200	0.150	0.317	0.305	0.196	0.179	0.146
- relative (% of body weight)	0.69	0.37	0.31	0.47	0.37	0.87	0.80	0.55	0.50	0.45
Lung tumours										
- adenoma	4	5	6	3	6	5	4	5	5	3
- carcinoma	3	0	0	1	0	0	0	0	1	0
- adenoma + carcinoma	7	5	6	4	6	5	4	5	6	3
Liver tumours										
- adenoma	9	13	8	6	5	0	0	1	1	0
- carcinoma	4	3	1	1	2	0	0	0	0	0
- adenoma + carcinoma	13	16	9	7	7	0	0	1	1	0

From Weiler (2000d)
* $P < 0.05$

The NOAEL for carcinogenicity was 25 000 ppm (equal to 3694 mg/kg bw per day for males and 4728 mg/kg bw per day for females), the highest dose tested. The NOAEL for toxicity was 2500 ppm (equal to 345 mg/kg bw per day for males and 441 mg/kg bw per day for females), based on reduced body weight and body weight gain at 25 000 ppm (Weiler, 2000d,e).

Rats

In a combined study of chronic toxicity and carcinogenicity conducted according to OECD Test Guideline No. 453, groups of 60 male and 60 female Crl:CD®(SD)BR VAF/Plus® rats were given diets containing dinotefuran (purity 93.0%) at a concentration of 0, 60, 200, 2000 or 20 000 ppm (equal to 0, 3, 10, 100 and 991 mg/kg bw per day for males and 0, 4, 13, 127 and 1332 mg/kg bw per day for females, respectively) for at least 104 weeks. Additional groups of 10 animals of each sex per group were similarly treated for at least 26, 52 and 78 weeks, and further groups of 10 animals of each sex treated at 0 and 20 000 ppm were treated for 26 weeks and then maintained untreated for 6 weeks before necropsy. Morbidity and mortality checks were performed twice daily, and a detailed clinical examination of all animals was performed weekly. Body weights and feed consumption were recorded weekly for 13 weeks and every 4 weeks thereafter. Ophthalmic examinations were performed on all animals pretest and after 26, 32, 78 and 104 weeks. Haematology, serum clinical chemistry and urine analysis were performed on 10 animals of each sex per group after 26, 52, 78 and 104 weeks and after 26 weeks of treatment for the animals treated at 0 and 20 000 ppm followed by 6

weeks treatment free. After 26, 52 and 78 weeks of treatment, 10 animals of each sex per group were killed. The additional 10 animals of each sex per group treated at 0 and 20 000 ppm for 26 weeks were killed after a 6-week treatment-free period. All other survivors were killed after at least 104 weeks of treatment. All decedents and scheduled kill animals were subjected to necropsy and postmortem examination of major organs and tissues. Selected organs were weighed from 10 animals of each sex per group, and samples of major organs and tissues were preserved from all animals. Samples of liver, kidney and adrenal were retained from two animals of each sex per group at each scheduled sacrifice for possible electron microscopic examination. All preserved tissues from the animals treated at 0 or 20 000 ppm and from decedents in all groups were examined by light microscopy. Gross lesions, endocrine glands, tissue masses, kidneys, liver, lungs and reproductive organs of both sexes were also examined from all animals in the intermediate-dose group.

Survival of both sexes to 104 weeks was unaffected by treatment at all dose levels. Control male survival in week 104 was 47% compared with 25–38% in the intermediate-dose groups and 53% in the high-dose dinotefuran group. Control female survival after 104 weeks was 33% compared with 30–43% in the dinotefuran-treated groups. There were no treatment-related clinical signs of toxicity, including the incidences of palpable tissue masses. There were no treatment-related ophthalmological findings at any dose level at any of the examination intervals. The body weight gains of both sexes were reduced by treatment at 20 000 ppm from week 2, but the effect was more severe in the females, with overall (weeks 1–104) body weight gains reduced by 5.5% and 44.1% in males and females, respectively. Body weight gain was unaffected by treatment at lower dose levels (Table 35). The mean weekly feed consumption of animals treated at 20 000 ppm was reduced by up to 10.0% during the first 77 weeks of treatment, but water intake was unaffected by treatment at all dose levels. Feed consumption was not affected by treatment at dose levels up to 2000 ppm (Table 35).

Table 35. Summary of selected findings in the 104-week rat study

	Males					Females				
	0 ppm	60 ppm	200 ppm	2000 ppm	20 000 ppm	0 ppm	60 ppm	200 ppm	2000 ppm	20 000 ppm
Body weight (g)										
- week 1	228	232	229	231	228	178	177	174	175	176
- week 26	685	694	692	702	638*	344	352	362*	350	314*
- week 50	794	802	815	822	722*	411	418	439*	418	347*
- week 78	836	841	865	867	777*	482	490	529*	497	377*
- week 105	758	792	808	800	729	553	608	565	545	417*
Body weight gain (g), weeks 1–104	531	565	585	574	504	376	433	395	370	242*
Feed consumption (g)										
- week 1	185	180	179*	181	167*	133	130	132	133	120*
- week 25	200	200	200	204	184*	145	145	146	146	133*
- week 49	205	201	204	213	189*	152	150	155	148	140*
- week 77	204	189	205	204	195	163	166	167	167	145*
- week 101	180	183	167	183	168	156	161	150	160	142
Kidney										
No. examined	*100*	*90*	*89*	*89*	*100*	*100*	*90*	*89*	*90*	*100*
- lymphohistiocytic infiltrate	42	51*	39	49*	65**	43	43	35	41	40
- tubular epithelial basophilia	42	47	37	51*	57*	48	42	34	40	26**

Table 35 (continued)

	Males					Females				
	0 ppm	60 ppm	200 ppm	2000 ppm	20 000 ppm	0 ppm	60 ppm	200 ppm	2000 ppm	20 000 ppm
- basement membrane thickened	30	35	32	44**	44*	23	24	19	19	15
- pelvic mineralization	5	5	4	7	27**	42	42	42	44	47
- chronic progressive nephropathy	35	26	36	24	14**	5	8	12*	8	0*
Thymus										
No. examined	*96*	*39*	*38*	*40*	*99*	*100*	*42*	*44*	*41*	*98*
- lymphocytic depletion	5	3	3	3	13*	9	4	2	2	11
Thyroid										
No. examined	*99*	*89*	*90*	*88*	*100*	*100*	*90*	*90*	*89*	*100*
- C-cell adenoma	8	12	10	12	17*	12	11	12	5	13
- C-cell carcinoma	1	0	0	0	0	0	0	1	1	1
- C-cell adenoma + carcinoma	9	12	10	12	17	12	11	13	6	14
Testes										
No. examined	*100*	*89*	*90*	*89*	*99*	—	—	—	—	—
- interstitial cell tumour	2	1	3	1	5	—	—	—	—	—
Uterus										
No. examined	—	—	—	—	—	*100*	*90*	*90*	*90*	*100*
- endometrial stromal polyps	—	—	—	—	—	1	0	3	3	6
Cervix										
No. examined	—	—	—	—	—	*100*	*40*	*43*	*40*	*100*
- endometrial stromal polyps	—	—	—	—	—	1	1	1	1	1
Vagina										
No. examined	—	—	—	—	—	*100*	*89*	*90*	*90*	*100*
- endometrial stromal polyps	—	—	—	—	—	0	0	1	1	0
Total (uterus + cervix + vagina)	—	—	—	—	—	2	1	5	5	7*
Mammary gland										
No. examined	—	—	—	—	—	*99*	*90*	*90*	*89*	*100*
- adenoma	—	—	—	—	—	11	11	7	9	9
- carcinoma	—	—	—	—	—	13	15	17	18	22
- adenoma + carcinoma	—	—	—	—	—	24	26	24	27	31

From Weiler (2000c)
* $P < 0.05$; ** $P < 0.01$

There were no treatment-related effects on haematology, serum clinical chemistry or urine analysis parameters at any dose level at any sampling interval. There were no treatment-related effects on the incidence of macroscopic findings at any necropsy interval. However, the mean number of uterine masses was higher than in the controls in the groups treated at 2000 and 20 000 ppm. There were 3, 1, 5 and 8 masses in the groups treated at 60, 200, 2000 and 20 000 ppm, respectively,

compared with 2 masses in the control group. The masses were commonly endometrial stromal polyps, but the incidence of the lesion and other uterine neoplasms was not significantly different from the controls ($P > 0.05$). There were no other notable differences in the incidence of macroscopic lesions between the treated and control groups. There were no treatment-related organ weight changes in either sex at any dose level after 26, 52, 78 and 104 weeks of treatment other than in females treated at 20 000 ppm, in which liver weight at week 79 was reduced as a consequence of growth retardation. There were no treatment-related effects on the nature and incidence of adverse non-neoplastic histopathological findings at any dose level. However, males treated at 20 000 ppm showed higher incidences of the renal changes pelvic mineralization, lymphohistiocytic infiltrate, tubular epithelial basophilia and thickening of the basement membrane. None of these renal changes was considered to be a treatment-related effect, as they either are common findings in rats or can be correlated with the lower incidence of chronic progressive nephropathy in males at 20 000 ppm. Similarly, the increased incidence of thymic lymphocyte depletion in males at 20 000 ppm was considered not to be a treatment-related effect, as these changes occur commonly in ageing rats.

There were no treatment-related effects at any dose level on the nature and incidence of tumours. However, pooled data from all animals showed differences between the control and 20 000 ppm group in the incidence of four tumour types. The incidence of thyroid C-cell adenomas was significantly higher in males treated at 20 000 ppm (17%) than in the controls (8%), but was within the historical control range of 1.7–24%. As this neoplasm is a common finding in the rat and because the total number of C-cell neoplasms (adenomas plus carcinomas) was not significantly higher than in the controls, this finding was considered not biologically relevant. Benign testicular interstitial cell tumours occurred at an incidence of 5.6% in animals treated at 20 000 ppm compared with a control incidence of 2.0%. As the difference from the controls was not statistically significant and the incidence was within the historical control range of 1.3–6.7%, the higher incidence is considered not to be treatment related. Benign endometrial stromal polyps occurred at a higher incidence in the uterus of animals treated at 20 000 ppm than in the controls, but the incidence in the uterus alone was not statistically significant. The combined incidence of this lesion in uterus, cervix and vagina at 20 000 ppm (7.0%) was significantly higher than the control incidence of 2.0%, but remained within the historical control range of 1.0–14%. As endometrial stromal polyps are considered a common spontaneous neoplasm, the slightly increased incidence at 20 000 ppm was considered not to be treatment related. Also, the incidence of mammary gland carcinomas was higher at 20 000 ppm (22%) than in the controls (13%), but was considered not to be treatment related, as it was not statistically significant and remained within the historical control range of 10.0–26.7%. With the exception of significantly lower incidences of pituitary adenomas in both sexes ($P < 0.05$) and adrenal phaeochromocytomas in males ($P < 0.01$), the incidences of all other tumours in the group treated at 20 000 ppm were not significantly different from the controls.

The NOAEL for carcinogenicity was 20 000 ppm (equal to 991 mg/kg bw per day for males and 1332 mg/kg bw per day for females), the highest dose tested. The NOAEL for toxicity was 2000 ppm (equal to 100 mg/kg bw per day for males and 127 mg/kg bw per day for females), based on a reduction in body weight, body weight gain and feed consumption at 20 000 ppm (Weiler, 2000c).

2.4 Genotoxicity

The results of the genotoxicity studies with dinotefuran are summarized in Table 36.

In a reverse gene mutation assay in bacteria conducted according to OECD Test Guideline No. 471, *Salmonella typhimurium* (strains TA98, TA100, TA1535, TA1537) and *Escherichia coli* (strain WP2*uvrA*) were exposed to dinotefuran (purity 96.5%) dissolved in dimethylsulfoxide (DMSO) in the presence and absence of S9 metabolic activation, using the preincubation method. Doses were selected based on the results of a range-finding study. For the main assay, doses of up to 5000 µg/plate were used, with three plates for each strain, condition and dose. Vehicle and positive controls were included in each experiment. In the main assay, dinotefuran did not influence the growth of any strain tested at dose levels of up to 5000 µg/plate. No appreciable increase in the reversion frequencies occurred in any strain tested in the dose range 313–5000 µg/plate. In contrast,

the positive control substances produced marked increases in the number of revertant colonies in all strains tested. Under the conditions of this study, dinotefuran did not induce gene mutations in the strains of *S. typhimurium* and *E. coli* used at doses of up to 5000 µg/plate (Takeda, 1996).

Table 36. Summary of genotoxicity studies with dinotefuran

End-point	Test object	Concentration or dose	Purity (%)	Result	Reference
In vitro					
Reverse mutation	*Salmonella typhimurium* (TA98, TA100, TA1535, TA1537) *Escherichia coli* (WP2*uvrA*)	± S9 mix: 0–5000 µg/plate	96.5	Negative	Takeda (1996)
DNA repair (rec-assay)	*Bacillus subtilis* (M45 *rec*⁻, H17 *rec*⁺)	± S9 mix: 0–16 000 µg/disc	96.5	Negative	Oguma (1999)
Gene mutation, *TK* locus	Mouse lymphoma L5178Y cells	± S9 mix: 0–2022 µg/ml	99.1	Negative	Lloyd (2002)
Chromosomal aberration	Chinese hamster lung (CHL) fibroblast cells	± S9 mix: 0–2000 µg/ml	96.5	Negative	Satou (1996)
In vivo					
Micronucleus induction	Male BDF1 (C57BL/6 × DBA/2) mice, bone marrow erythroblasts	0, 270, 540 and 1080 mg/kg bw; twice (24 h apart); oral administration	99	Negative	Nakajima (1995)

DNA, deoxyribonucleic acid; S9, 9000 × *g* supernatant fraction of rat liver homogenate

In a DNA repair assay (rec-assay) in bacteria conducted according to JMAFF test guideline 59 NohSan No. 4200 (1985), *Bacillus subtilis* strains M45 (*rec*⁻) and H17 (*rec*⁺) were exposed to dinotefuran (purity 96.5%) dissolved in DMSO in the presence and absence of S9 metabolic activation. Doses were selected based on the results of a range-finding study. For the main assay, doses of up to 16 000 µg/plate were used, with two plates for each strain, condition and dose. Vehicle (DMSO), negative controls (kanamycin or streptomycin) and positive controls (mitomycin C or 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole acetate) were included in each experiment. In the main assay, DMSO solvent alone and all doses of dinotefuran employed, up to and including the highest dose, 16 000 µg/plate, produced no growth inhibition of either strain of *B. subtilis* with or without S9 metabolic activation. The negative control substances without and with S9 produced differences in the inhibition zone diameters of 1.7 and 1.1 mm, respectively, whereas the positive control substances without and with S9 produced differences of 7.2 and 6.0 mm, respectively. Under the conditions of the study, dinotefuran did not exhibit DNA-damaging activity in *B. subtilis* at doses of up to 16 000 µg/plate (Oguma, 1999).

In an in vitro mammalian cell gene mutation test conducted according to OECD Test Guideline No. 476, dinotefuran (purity 99.1%) was tested for its ability to induce forward mutations at the *TK* locus in mouse lymphoma L5178Y cells. Two independent sets of experiments were conducted in the presence and absence of S9 metabolic activation. The highest dose levels for the main assay, equivalent to 10 mmol/l, were determined on the basis of two preliminary cytotoxicity tests. In experiment 1, duplicate cultures were exposed for 3 hours to dinotefuran in saline at a final concentration of 0 (saline), 400, 800, 1200, 1600 or 2022 µg/ml, both with and without metabolic activation. In experiment 2, the same concentrations were assayed without metabolic activation for a 24-hour exposure period or with metabolic activation for a 3-hour exposure period. Concurrent positive controls with and without S9 were also tested. After the exposure period, cells were washed

and cultures were transferred to flasks for a 2-day mutation expression period. Then, cultures were incubated for 12 days in selection medium containing trifluorothymidine at 3 μg/ml. Wells containing large and small colonies were enumerated for the negative and positive controls. In both experiments, relative survival at the highest concentration tested was at least 76% with and without metabolic activation. No statistically significant increases in mutation frequency occurred at any concentration in the absence or presence of metabolic activation in either independent experiment. Marked increases in the numbers of both small and large colony mutants occurred in response to both positive control materials. Under the conditions of the study, dinotefuran did not induce mutation at the *TK* locus of L5178Y mouse lymphoma cells at concentrations of up to 10 mmol/l in the presence and absence of S9 metabolic activation (Lloyd, 2002).

In an in vitro mammalian chromosomal aberration test conducted according to OECD Test Guideline No. 473, the clastogenic potential of dinotefuran (purity 96.5%) was tested in Chinese hamster lung (CHL/IU) cells. Concentrations for the main assay were determined following a preliminary cytotoxicity test, and the high concentration selected was 2000 μg/ml (10 mmol/l). A solvent control (saline) and a series of three concentrations (500, 1000 and 2000 μg/ml) were used for each of the four treatment regimens: 6-hour exposure without S9, 6-hour exposure with S9 and 24-hour or 48-hour exposure without S9. All dose levels were evaluated for clastogenicity by evaluating 200 metaphase cells per concentration. Cell survival was determined at harvest. Mitomycin C (0.03 μg/ml) was the positive control for the 24-hour and 48-hour exposures without activation, and cyclophosphamide (12 μg/ml) was the positive control for the 6-hour exposures with and without activation. Structural or numerical aberrations were not induced by dinotefuran after either 24 or 48 hours of exposure to concentrations up to 2000 μg/ml. However, slight growth inhibition occurred at 2000 μg/ml, 28% after the 24-hour exposure and 38% after the 48-hour exposure. Similarly, structural or numerical aberrations were not induced after 6 hours of exposure to dinotefuran in either the presence or absence of metabolic activation. Cell survival was unaffected at all concentrations after 6 hours of exposure. Both mitomycin C and cyclophosphamide produced marked increases in the incidences of structural aberrations, notably chromatid breaks and exchanges. Under the conditions of this study, dinotefuran did not induce structural or numerical chromosomal aberrations in Chinese hamster lung cells at concentrations of up to 2000 μg/ml (Satou, 1996).

In a mammalian erythrocyte micronucleus test conducted according to JMAFF test guideline 59 NohSan No. 4200 (1985), groups of six male BDF1 (C57BL/6 × DBA/2) mice received two oral (gavage) doses (24 hours apart) of dinotefuran (purity 99%) at 0 (0.5% aqueous carboxymethylcellulose), 270, 540 or 1080 mg/kg bw in a volume of 20 ml/kg bw. The dose levels were based on the results of a range-finding study in which groups of six male mice were treated once daily, by oral gavage, for 2 days with dinotefuran at a dose level of 648, 1080, 1800, 3000 or 5000 mg/kg bw. The vehicle served as negative control, and mitomycin C (2 mg/kg bw, intraperitoneally) as positive control. The animals were terminated 24 hours after the last administration, the bone marrow of the femur was prepared, and 1000 polychromatic erythrocytes were evaluated per animal and investigated for micronuclei. The ratio of polychromatic erythrocytes to normochromatic erythrocytes was determined in 1000 erythrocytes. In the range-finding study, all mice treated at 3000 or 5000 mg/kg bw and four of the six mice treated at 1800 mg/kg bw died, and the mean incidences of micronucleated polychromatic erythrocytes were similar between treated groups and between the different sampling time points (24, 48 and 72 hours after dosing). In the main study, there were no deaths during the treatment period, and no signs of toxicity, including body weight change, were evident at any dose level. The micronucleated polychromatic erythrocyte frequencies and polychromatic erythrocyte/total erythrocyte ratios of all dinotefuran-treated groups were comparable to and not significantly different from control values. In contrast, mitomycin C produced a statistically significant increase in the micronucleated polychromatic erythrocyte frequency and a statistically significant decrease in the polychromatic erythrocyte/total erythrocyte ratio. Under the conditions of this study, dinotefuran did not induce the formation of micronuclei in bone marrow erythroblasts of mice after two oral doses of up to 1080 mg/kg bw (Nakajima, 1995).

2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

In a dose range–finding reproductive toxicity study, groups of six male and six female Hanlbm: WIST (SPF) rats were given diets containing dinotefuran (purity 98.9%) at a concentration of 0, 10 000 or 20 000 ppm for 2 weeks before mating and during the mating period. The substance intakes at 10 000 and 20 000 ppm during the premating period were equal to 700 and 1340 mg/kg bw per day for males and 779 and 1507 mg/kg bw per day for females, respectively. P generation males continued to be treated until necropsy, shortly before weaning of the F_1 litters, whereas P generation females continued to be treated throughout gestation and lactation until necropsy shortly after weaning of the F_1 litters. Pregnant females were allowed to litter normally and rear their young to weaning. Litters were culled on day 4 to four pups of each sex, where possible. After weaning (day 21), six F_1 animals of each sex per group were treated at the same dietary concentrations as the P generation for a further 2 weeks. The substance intakes at 10 000 and 20 000 ppm from weaning were at least 745 and 1585 mg/kg bw per day for males and 770 and 1796 mg/kg bw per day for females, respectively.

P generation animals were checked at least twice daily for morbidity and mortality and for clinical signs of a reaction to treatment. Body weights were recorded weekly except during pairing. After mating, females were weighed on days 0, 7, 14 and 21 of gestation and days 1, 4, 7, 14 and 21 postpartum. Feed consumption was measured weekly throughout the study until day 14 postpartum, except during mating. Towards the end of gestation, females were examined twice daily for evidence of parturition. Day 0 of lactation was the day of completion of parturition. P generation animals were subjected to necropsy and postmortem examination. Implantation sites were counted in dams with litters, and uteri were placed in ammonium sulfide solution to aid the visualization of implantation sites. Major organs, including testes/ovaries, were preserved from P generation animals, weaned F_1 pups and F_1 pups selected for further study. An epididymal sperm sample was obtained from all P generation males, and one epididymis and one testis were retained for the determination of homogenization-resistant spermatids and caudal epididymal sperm reserve. Ovarian histopathology comprising quantitative primordial follicle counts in 10 levels per ovary and a comparison with secondary/tertiary follicles was performed on all females treated at 0 or 20 000 ppm.

Litters were examined as soon as possible after birth for litter size, live births, stillbirths and gross abnormalities. The sex ratio was determined on days 0, 4 and 21 of lactation. Pups were weighed on days 0/1, 4, 7, 14 and 21 of lactation. Dams and pups were observed daily for survival and abnormalities in nesting or nursing behaviour. F_1 animals selected for 2 weeks of further treatment were weighed on days 24, 28 and 35 postpartum, and feed consumption was recorded at 4- or 6-day intervals. Dead pups, except where excessively cannibalized, were subjected to necropsy. All F_1 pups were culled on day 4 postpartum, and pups not selected for further study were culled on day 21; pups selected for 2 weeks of further treatment were subjected to necropsy and examined for gross abnormalities.

There were no deaths in the P generation at any dose level, and treatment-related clinical signs were confined to soft faeces in females at 20 000 ppm during lactation. There was a treatment- and dose-related decrease in feed consumption and body weight gain throughout the treatment period in females treated at 10 000 and 20 000 ppm. Thus, the group mean body weights were significantly lower than those of the control group at the end of the prepairing, gestation and lactation periods. Males treated at 20 000 ppm also showed reduced body weight gain and significantly reduced feed consumption during the 1st week of treatment (Table 37).

Table 37. Summary of selected findings in the dose range–finding reproductive toxicity study in rats

	Males			Females		
	0 ppm	10 000 ppm	20 000 ppm	0 ppm	10 000 ppm	20 000 ppm
P generation						
Body weight gain (g)						
- weeks 1–2	36	25	14	16	11	6
- weeks 3–7	54	45	41	—	—	—
- gestation	—	—	—	122	102	82
- lactation	—	—	—	52	26	7
Feed consumption (g/day)						
- week 1	24.4	22.6	20.3*	18.6	15.4**	14.6**
- week 2	24.8	23.6	21.6	18.4	16.7**	16.1*
- weeks 3–7	24.1	24.2	22.3	—	—	—
- gestation	—	—	—	21.3	18.9	19.6
- lactation	—	—	—	43.8	40.4	32.9
F_1 generation (after weaning)						
Body weight (g)						
- day 1	48	35**	31**	46	34**	29**
- day 14	118	95*	76**	99	84*	70**
Body weight gain (g), days 1–14	70	60	45	53	50	41
Feed consumption (g/day)						
- days 1–4	7.0	3.2**	2.9**	6.3	3.1**	2.8**
- days 4–8	11.9	10.2	8.3**	10.6	9.6	8.2**
- days 8–14	15.8	12.6*	11.0**	13.3	12.0	10.2**

From Edwards, Knuppe & Weber (2001)
* $P < 0.05$; ** $P < 0.01$

Treatment-related gross findings at necropsy in the P generation were confined to an increased incidence of small thymus in females at 20 000 ppm (incidences were 2/6, 3/6 and 6/6 in the groups treated at 0, 10 000 and 20 000 ppm, respectively). There were no treatment-related gross findings at necropsy in the surplus F_1 generation pups culled on day 4 or 21 postpartum or in animals selected for 2 weeks of treatment after weaning, although one male animal at 20 000 ppm died in the 1st week after selection. There were no treatment-related effects at either dose level in P generation males on sperm motility (% progressively motile sperm), sperm morphology or sperm counts (homogenization-resistant spermatids and caudal epididymal sperm counts). There was no treatment-related effect on the number and distribution of ovarian follicle types in females treated at 20 000 ppm. Although the chi-squared test revealed a significant ($P < 0.001$) difference between the control and treated groups in the distribution of follicle types, the difference was due to a slightly higher number of primordial follicles in the treated group, which is considered to be within the normal range of variation. Moreover, the *t*-test revealed negative results only. Therefore, dinotefuran was considered not to have altered ovarian anatomy at a dose level of 20 000 ppm (Table 38).

Table 38. Summary of selected reproductive findings in the dose range–finding reproductive toxicity study in rats

	0 ppm	10 000 ppm	20 000 ppm
Ovary			
- no. of primordial follicles	749	NE	877
- no. of growing primordial follicles	616	NE	665
- no. of secondary/tertiary follicles	1151	NE	1149
Mean duration of gestation (days)	21.5	22.0	22.0
Total no. of litters born	6	6	6
Mean no. of implantations/dam	14.5	12.0	11.5*
Birth index[a]	95.4	95.8	79.7**
Post-implantation loss, total (% implantations)	4 (4.6)	3 (4.2)	14** (20.3)
Live pups at birth (mean/dam)	13.8	11.5	9.2*
Dead pups at birth (mean/dam)	0.0	0.0	0.0
Mean postnatal loss, days 0–4 (mean/dam)	0.3	0.0	0.2
Mean no. of live pups on day 4	8.0	8.0	7.5
Viability index[b]	97.6	100.0	98.2
Postnatal loss, days 5–21 (mean/dam)	0.2	0.0	0.5
Mean no. of live pups on day 21	7.8	8.0	7.0
Weaning index[c]	97.9	100.0	93.3
Sex ratio (% males)	49	54	48
Pup body weights (g), males + females			
- day 0	5.1	5.3	5.3
- day 4	7.7	7.9	8.1
- day 7	12.9	12.3	11.5
- day 14	29.5	22.4*	19.4**
- day 21	46.8	35.0*	29.0**

From Edwards, Knuppe & Weber (2001)
NE, not evaluated; * $P < 0.05$; ** $P < 0.01$
[a] No. of liveborn pups × 100 / no. of implantations.
[b] No. of pups alive on day 4 × 100 / no. of liveborn pups.
[c] No. of pups alive on day 21 × 100 / no. of pups alive on day 4.

There was no effect of treatment on fertility or mating performance. All females in all experimental groups mated successfully and reared pups to weaning. The median precoital time was comparable in the treated and control groups. There was a treatment-related decrease in the number of implantations and increased post-implantation loss, leading to a significantly reduced mean litter size at birth, at 20 000 ppm. Although the mean number of implantations and litter size at birth at 10 000 ppm were lower than control values, a treatment effect was not indicated, because neither parameter was statistically significant or the control values were higher than normal (Table 38).

Neonatal and preweaning viability, the weaning indices and sex ratios at 10 000 and 20 000 ppm were not significantly different from those of the control group. There was a treatment- and dose-related decrease in the preweaning body weight gain of male and female pups at 10 000 and 20 000 ppm. The effect was statistically significant from day 14 postpartum, and at weaning on day 21, the group mean pup weights for the sexes combined were reduced by 25.2% and 38.0%, respectively (Table 38).

The weight gain of F_1 generation animals treated for 2 weeks after weaning continued to be depressed in both sexes at both dose levels. The feed consumption of both sexes at both dose levels was also markedly depressed, particularly during the first 4 days of treatment.

No NOAEL for parental toxicity and offspring toxicity could be identified, because reduced body weight, body weight gain and feed consumption were observed at all dose levels. The LOAEL was 10 000 ppm (equal to 700 mg/kg bw per day). The NOAEL for reproductive toxicity was 10 000 ppm (equal to 700 mg/kg bw per day), based on a reduced number of implantations, an increase in post-implantation loss and a reduced litter size at birth at 20 000 ppm (equal to 1340 mg/kg bw per day) (Edwards, Knuppe & Weber, 2001).

In a two-generation reproductive toxicity study conducted according to OECD Test Guideline No. 416, groups of 25 male and 25 female Hanlbm: WIST (SPF) rats were given diets containing dinotefuran (purity 98.9%) at a concentration of 0, 300, 1000, 3000 or 10 000 ppm (equal to 0, 24.1, 79.9, 241 and 822 mg/kg bw per day for P generation males [premating period] and 0, 26.8, 90.1, 268 and 907 mg/kg bw per day for P generation females [premating period] for 10 weeks prior to mating through to weaning of the F_1 offspring. Groups of 25 male and 25 female F_1 generation offspring were then similarly treated; the mean substance intakes at 0, 300, 1000, 3000 and 10 000 ppm were equal to 0, 27.2, 90.5, 269 and 935 mg/kg bw per day for F_1 generation males (premating period) and 0, 29.6, 96.5, 293 and 1005 mg/kg bw per day for F_1 generation females (premating period). In P and F_1 parental animals, clinical signs were recorded daily, body weight and feed consumption were recorded approximately weekly, estrous cyclicity was monitored, the duration of gestation was recorded and the F_1 animals were examined for sexual development landmarks. Pregnant females were allowed to litter normally, and the litters were examined for live births, stillbirths and external abnormalities. The sexes and body weights of pups were recorded. Litters were not standardized by culling. Anogenital distance was measured in all F_2 generation pups on day 1 of lactation. At 6 weeks of age, F_1 animals selected for functional investigations (20 animals of each sex per group) were subjected to assessment of behavioural function, grip strength and locomotor activity.

All surviving and decedent P and F_1 parental animals were subjected to necropsy and gross pathological examination. Analyses for sperm motility, morphology and epididymal counts and quantitative primordial ovarian follicle counts were performed. Major organs, including reproductive organs, were weighed. Histopathological examination of reproductive organs, pituitary and adrenal glands was performed on all P and F_1 generation parental animals treated at 0 or 10 000 ppm. Additional testicular histopathology, qualitative sperm staging, was performed. Histopathology was also performed on the reproductive organs of animals treated at 300, 1000 or 3000 ppm that failed to mate.

Unselected F_1 weanlings, decedent F_1 offspring and weaned F_2 offspring were subjected to gross necropsy. One male and one female weanling per litter per generation were subjected to organ weight analysis of brain, spleen, thymus and uterus. Reduced-weight thymus and spleen of F_1/F_2 weanlings were examined microscopically.

P/F_1 parental animals: One P generation female at 10 000 ppm died prematurely on day 21 postpartum after showing clinical signs of soft faeces, ruffled fur and blood-stained urine during lactation. Macroscopic and histopathological examination showed renal changes that were considered causal of death. Although death may have been incidental to treatment with dinotefuran, a treatment-related etiology cannot be precluded because of its occurrence at the highest dose level at a time of greatly increased dosage. All other P and F_1 generation parental animals survived the scheduled treatment period. Treatment-related clinical signs were confined to soft faeces during lactation in all P generation females at 10 000 ppm and one F_1 generation female treated at 10 000 ppm. The observation was considered to be treatment related, as its occurrence was confined to the lactation period, at which time high dose levels were ingested. No treatment-related clinical signs occurred in males at 10 000 ppm or in either sex at lower dose levels in either parental generation.

Minor and transient effects on feed consumption occurred during the prepairing period, which were suggestive of reduced diet palatability. P and F_1 generation parental animals of both sexes showed significantly reduced feed consumption during weeks 1 and/or 2 of treatment. P generation males at 300 and 1000 ppm also showed slightly lower feed consumption compared with the controls during the 1st week of treatment only. Thereafter, there was no clear treatment-related effect on the feed consumption of either sex at any dose level during the prepairing period. The feed consumption of the P generation females during gestation was not affected by treatment at any dose level, but was significantly reduced by 11.3% during the 1st week of gestation in F_1 generation females at 10 000 ppm. During lactation, the feed consumption in P and F_1 generation females treated at 3000 and 10 000 ppm was slightly reduced, but as the effect at 3000 ppm was not accompanied by an effect on body weight gain, the effect was considered not to be adverse at 3000 ppm (Table 39).

The group mean body weight gains during the prepairing period were reduced in P generation males and females at 10 000 ppm. Thus, group mean body weights at the start of mating were 5.8% and 4.0% lower than those of the controls, respectively. The treatment-related reduction in preweaning body weight gains of F_1 generation males and females at 10 000 ppm persisted during the prepairing treatment period. Thus, group mean body weights at the start of mating were 9.3% and 4.3% lower than the control values, respectively. The body weight gains of P and F_1 generation animals of both sexes at 300–3000 ppm were unaffected by treatment with dinotefuran (Table 39).

The slightly lower body weights of P and F_1 generation females at 10 000 ppm persisted during the gestation and lactation periods. The group mean body weights at 21 days postpartum were 6.4% and 7.9% lower, respectively, than control values. The body weights of P and F_1 generation females treated at 300–3000 ppm were comparable to control values throughout gestation and lactation (Table 39).

There was no effect of treatment at any dose level in either generation on the duration of the estrous cycle. There were no treatment-related effects at any dose level in either generation on fertility and mating performance, duration of gestation, number of implantations, post-implantation loss, litter size at birth, pup mortality, litter size at weaning or sex ratio. With the exception of two non-dose-related occurrences of statistical significance in the F_1 generation (higher neonatal pup mortality at 300 ppm and higher number of empty implantation sites at 3000 ppm), all reproductive data in the treated groups were comparable to, and not significantly different from, the control group values (Table 40).

There were no treatment-related effects on sperm motility, morphology or counts in either P or F_1 generation males at any dose level. The proportions of non-motile, stationary and progressively motile sperm in all treated P generation groups were similar to, and not significantly different from, the control group values. In the F_1 generation, statistically significant variation from the control values was observed for progressively motile and stationary sperm in the group treated at 10 000 ppm and for progressively motile sperm in the group treated at 1000 ppm. Males at 10 000 ppm showed 40% stationary and 50% progressively motile sperm compared with the control group, which showed 32% stationary and 58% progressively motile sperm. The proportion of non-motile sperm in both groups was 10%. The group treated at 1000 ppm also showed a significantly lower proportion of progressively motile sperm. As the differences in the mean values were numerically small and showed no clear dose dependency, the small differences recorded are considered to be incidental to treatment. P generation males at 10 000 ppm showed a slightly, but significantly, higher incidence of sperm abnormality type D (normal head but abnormally curved hook). However, the finding occurred in association with a high percentage incidence of normal sperm (95.0% versus 96.2% in the control), and the numerical difference from the control for this abnormality (type D) was small (2% affected versus 1.1% in the control). Furthermore, the F_1 generation control incidence of type D abnormality was 1.7%. Therefore, the higher incidence in the P generation males at 10 000 ppm was considered to be incidental to treatment. In the F_1 generation, there were minor differences from the control in the sperm morphology data, but the differences occurred in association with a high percentage incidence of normal sperm (95.4% versus 96.4% in the control), and the differences were considered to be incidental to treatment. There was no significant effect on epididymal sperm count for the P or F_1 generation males, but the testicular sperm counts of the P generation males showed a slight reduction

at 10 000 ppm, which was statistically significant. However, as the magnitude of the difference was small, occurred in the absence of a significant effect on epididymal sperm count and was not repeated in the F_1 generation males, the difference was considered incidental to treatment.

Table 39. Summary of selected parental findings in the two-generation rat study

	Males					Females				
	0 ppm	300 ppm	1000 ppm	3000 ppm	10 000 ppm	0 ppm	300 ppm	1000 ppm	3000 ppm	10 000 ppm
P generation										
Body weight (g)										
- week 1	145	145	143	145	144	112	113	109	110	109
- week 10	397	391	384	380	374*	225	227	226	223	216
- week 14	450	443	436	431	424*	—	—	—	—	—
- GD 0	—	—	—	—	—	224	225	225	222	217
- GD 7	—	—	—	—	—	245	243	244	240	233*
- GD 21	—	—	—	—	—	338	332	331	327	317**
- LD 1	—	—	—	—	—	245	244	246	243	230*
- LD 21	—	—	—	—	—	282	281	282	274	264**
Feed consumption (g/day)										
- week 1	22.9	21.9	21.0**	21.7*	20.6**	15.7	16.2	15.3	15.5	14.6**
- week 2	23.3	22.9	22.4	22.9	22.5	16.3	16.4	16.1	15.9	15.3*
- weeks 1–10	24.4	24.1	23.4	23.7	23.8	17.0	16.8	16.7	16.5	16.4
- gestation	—	—	—	—	—	21.3	20.2	20.9	20.7	20.4
- lactation	—	—	—	—	—	44.7	43.3	44.2	41.7	41.2
F_1 generation										
Body weight (g)										
- week 1	61	62	64	65	52*	58	56	60	59	52*
- week 10	344	347	351	344	312*	207	207	211	209	198
- week 18	421	423	427	422	387*	—	—	—	—	—
- GD 0	—	—	—	—	—	211	208	216	211	203
- GD 7	—	—	—	—	—	229	227	234	230	220
- GD 21	—	—	—	—	—	319	319	322	315	307
- LD 1	—	—	—	—	—	233	230	235	232	225
- LD 21	—	—	—	—	—	280	275	279	269	258**
Feed consumption (g/day)										
- week 1	12.8	13.0	13.3	13.6	11.3	12.2	11.7	12.2	12.2	11.2
- week 2	17.4	17.8	18.0	17.2	14.6**	15.1	14.7	15.2	14.0*	13.3**
- weeks 1–10	20.3	21.3	21.4	21.2	19.4	14.8	15.5	15.5	15.4	15.0
- gestation	—	—	—	—	—	19.6	18.5	18.9	18.6	18.5
- lactation	—	—	—	—	—	41.2	40.4	42.2	40.6	40.0

From Becker (2002)

GD, gestation day; LD, lactation day; * $P < 0.05$; ** $P < 0.01$

Table 40. Summary of selected reproductive data in the two-generation rat study

	0 ppm	300 ppm	1000 ppm	3000 ppm	10 000 ppm
P generation					
No. paired / no. mated	25 / 25	25 / 25	25 / 25	25 / 25	25 / 25
No. pregnant	24	25	25	25	24
No. with viable litters	23	25	22	25	23
Duration of estrous cycle (days)	5.3	5.6	4.8	5.1	4.9
Mean precoital time (days)	2.8	2.8	2.8	2.6	2.6
Duration of gestation (days)	21.5	21.4	21.5	21.5	21.8
No. of implantations/dam	13.1	13.2	12.6	12.5	12.9
Post-implantation loss (%)	7.3	7.9	9.7	9.3	7.1
Live litter size on day 0/1	12.2	12.2	11.4	11.3	12.0
Pup mortality (group total) on days 0–4 postpartum	5	17*	6	4	7
Litter size on day 4 postpartum	12.0	11.5	11.1	11.2	11.6
Pup mortality (group total) on days 5–21 postpartum	6	4	2	0	7
Litter size on day 21 postpartum	11.7	11.4	11.0	11.2	11.3
Sex ratio (% males)	51	49	47	47	45
F_1 generation					
No. paired / no. mated	25 / 25	25 / 25	25 / 25	25 / 25	25 / 25
No. pregnant	25	24	24	25	25
No. with viable litters	25	24	24	25	25
Duration of estrous cycle (days)	4.9	4.9	4.9	5.0	5.0
Mean precoital time (days)	2.7	2.8	2.5	2.6	2.4
Duration of gestation (days)	21.5	21.4	21.5	21.5	21.5
No. of implantations/dam	11.8	11.8	11.5	11.4	12.3
Post-implantation loss (%)	6.4	3.5	4.0	10.9	5.2
Live litter size on day 0/1	11.1	11.4	11.0	10.2	11.6
Pup mortality (group total) on days 0–4 postpartum	5	6	1	1	5
Litter size on day 4 postpartum	10.9	11.1	11.0	10.1	11.4
Pup mortality (group total) on days 5–21 postpartum	2	2	1	2	0
Litter size on day 21 postpartum	10.8	11.0	11.0	10.0	11.4
Sex ratio (% males)	52	46	49	55	46

From Becker (2002)
* $P < 0.05$; ** $P < 0.01$

There were no treatment-related gross findings at necropsy in the male and female P and F_1 generation parental animals at any dose level, but significantly reduced spleen weights (absolute and brain weight ratios, 10.0–16.0% reduced) occurred in both sexes of the P generation treated at 10 000 ppm. The effect was not evident at lower dose levels or in F_1 generation parental animals at any dose level. Female F_1 generation parental animals at 10 000 ppm showed significantly reduced thyroid weights (absolute, body weight and brain weight ratios, 20.0–25.0% reduced). Other minor, but statistically significant, differences in organ weights at 10 000 ppm were considered to be secondary

to lower terminal body weights. All histopathological findings recorded in the reproductive organs, pituitary and adrenal glands of P and F_1 generation males and females were considered to be within the range of background lesions commonly observed in rats of this strain and age. The incidences of all individual findings at 10 000 ppm did not indicate an effect of treatment. There were no treatment-related findings during staging analysis of the testes. All cycles were complete, and there were no indicators for maturation arrest. There were no treatment-related, biologically relevant effects on the quantitative evaluation of ovarian follicular stages. Although ovary staging revealed a significantly lower number of primordial follicles in 10 000 ppm animals, the finding was deemed to have no biological significance, because the numbers of antral follicles were markedly higher at 10 000 ppm, and the numbers of corpora lutea were also slightly higher than the control values. The numbers of pre-antral follicles were comparable in the treated and control groups.

F_1/F_2 offspring: There were no treatment-related effects at any dose level in either generation on the nature and incidence of pup abnormalities during the preweaning period. The anogenital distance of F_2 progeny of both sexes was unaffected by treatment at all dose levels. The significantly greater anogenital distances of F_2 pups at 1000 ppm are considered incidental to treatment, as a dose–response relationship was not evident. Preweaning pup growth in both the F_1 and F_2 generations was retarded at 10 000 ppm. Group mean male and female pup weights were significantly reduced from day 14 postpartum, except for female F_1 pups, for which weights were significantly reduced on day 21 postpartum only. Thus, at weaning, pup weights were 11.6–15.1% lower than control values. Pup weights were unaffected by treatment at lower dose levels (Table 41).

Sexual maturation of F_1 generation pups, based on preputial separation or vaginal patency, was unaffected by treatment at all dose levels. The group mean age at which these events occurred was 27.9, 28.0, 27.8, 28.2 and 28.2 days (males) and 34.3, 34.1, 34.5, 33.9 and 35.5 days (females), in order of ascending dose level. Quantitative locomotor activity of F_1 progeny at 6 weeks of age was not affected by treatment at any dose level. There were no statistically significant differences in the low beam counts recorded for control and treated groups of either sex. There were no treatment-related effects at any dose level on motor capability as assessed by grip strength. Quantitative measurement of grip strength showed significantly lower absolute values at 10 000 ppm for male forelimb grip strength and female hindlimb grip strength. However, grip strength to body weight ratios were not significantly different from those of the controls, suggesting that the differences were due to lower body weight or smaller size at 10 000 ppm, rather than a specific effect of dinotefuran on motor capability. Absolute grip strength at 3000 ppm and below was not affected by treatment. None of the animals at any dose level showed any behavioural, postural, motor, respiratory or reflex anomalies in the modified Irwin screen, and all animals were of normal appearance (Table 41).

The types and frequencies of gross lesions at necropsy in F_1 and F_2 pups shortly after weaning gave no indication of treatment-related effects. The most common finding in both generations was renal pelvic dilatation, but the group incidences did not indicate an effect of treatment. The overall incidences of renal pelvic dilatation were 11.0%, 7.7%, 11.5%, 14.3% and 10.0% (F_1 generation) and 8.0%, 2.1%, 17.0%, 12.5% and 4.1% (F_2 generation), in order of ascending dose level. All other gross lesions occurred at very low incidences, and their distribution did not suggest an effect of treatment. Direct treatment-related effects on organ weights were confined to the spleen in both the F_1 and F_2 generations. The mean absolute spleen weight (both sexes) and mean spleen weight relative to body weight (females only) were significantly reduced by up to 25.6% in F_1 generation pups treated at 10 000 ppm. The mean brain weight relative to body weight was also significantly elevated in these animals, but this is considered to be due to the lower body weights of the group. There was no effect on thymus weights at any dose level. Absolute and relative spleen weights were significantly reduced to a similar extent in both sexes of the F_2 generation at 10 000 ppm. Absolute brain and thymus weights were significantly reduced and relative brain weights were significantly increased in F_2 animals exposed to 10 000 ppm. However, the pattern of response was indicative of a body weight effect rather than a specific effect of dinotefuran on these organs. There were no effects on any of the measured organ weights in F_1 and F_2 generation male and female pups at 300–3000 ppm (Table 41).

Table 41. Summary of selected offspring findings in the two-generation rat study

	Males					Females				
	0 ppm	300 ppm	1000 ppm	3000 ppm	10 000 ppm	0 ppm	300 ppm	1000 ppm	3000 ppm	10 000 ppm
F_1 generation										
Body weight (g)										
- day 0/1	5.7	5.4	5.7	6.0	5.5	5.4	5.1	5.8	5.6	5.5
- day 4	8.3	8.1	8.5	8.7	8.2	8.0	7.6	8.3	8.2	8.0
- day 7	12.3	12.0	12.8	12.9	11.7	11.9	11.4	12.6	12.3	11.4
- day 14	23.9	24.0	25.0	24.7	21.2**	23.4	22.7	24.5	23.7	21.3
- day 21	37.4	37.3	38.8	38.1	32.0**	36.2	35.3	38.2	36.6	32.0*
Spleen weight, day 21										
- absolute (g)	0.15	0.14	0.17	0.16	0.12*	0.16	0.15	0.18	0.16	0.12**
- relative (% of body weight)	0.41	0.38	0.44	0.43	0.37	0.44	0.42	0.46	0.44	0.37**
Forelimb grip strength, week 6										
- absolute (g)	618	576	557	554	505*	536	513	504	521	449
- grip strength/ body weight ratio	3.37	3.21	3.05	3.05	3.23	3.93	3.85	3.62	3.88	3.64
Hindlimb grip strength, week 6										
- absolute (g)	387	416	350	379	343	428	401	364*	385	336*
- grip strength/ body weight ratio	2.12	2.30	1.94	2.09	2.21	3.15	3.03	2.60*	2.88	2.73
F_2 generation										
Body weight (g)										
- day 1	5.9	5.9	6.2	6.2	5.9	5.6	5.6	5.8	5.8	5.7
- day 4	8.7	8.6	9.0	9.2	8.5	8.3	8.4	8.6	8.7	8.3
- day 7	12.7	12.4	13.2	13.5	12.1	12.2	12.2	12.7	12.8	12.0
- day 14	24.7	24.9	25.6	25.8	21.5**	24.2	24.4	24.7	24.7	21.2**
- day 21	39.7	39.5	41.7	41.4	33.7**	38.7	38.8	40.1	39.6	33.1**
Anogenital distance (mm), day 1	2.12	2.17	2.24**	2.16	2.12	1.00	1.00	1.04*	1.02	1.01
Spleen weight, day 21										
- absolute (g)	0.17	0.17	0.19	0.19	0.13**	0.17	0.16	0.19	0.18	0.13**
- relative (% of body weight)	0.43	0.43	0.45	0.45	0.37**	0.45	0.41	0.47	0.45	0.39*

From Becker (2002)
* $P < 0.05$; ** $P < 0.01$

The NOAEL for reproductive toxicity was 10 000 ppm (equal to 822 mg/kg bw per day for males and 907 mg/kg bw per day for females), the highest dose tested. The NOAEL for parental toxicity was 3000 ppm (equal to 241 mg/kg bw per day for males and 268 mg/kg bw per day for females), based on a reduction in feed consumption, body weight and spleen weight at 10 000 ppm.

The NOAEL for offspring toxicity was 3000 ppm (equal to 241 mg/kg bw per day for males and 268 mg/kg bw per day for females), based on reduced pup weight gain during lactation and reduced spleen weight at 10 000 ppm (Becker, 2002).

(b) Developmental toxicity

Rats

In a range-finding prenatal developmental toxicity study, groups of six mated female Crj:CD(SD) IGS rats were administered dinotefuran (purity 92.9%) in 0.5% aqueous carboxymethylcellulose at a dose volume of 10 ml/kg bw by oral gavage at a dose level of 0, 30, 100, 300 or 1000 mg/kg bw per day from gestation day (GD) 6 to GD 15. Clinical signs, body weight and feed consumption were recorded. On GD 20, dams were sacrificed and the fetuses delivered by caesarean section. The uterine tract and ovaries were removed, and pregnancy was confirmed. If implantations were not visible macroscopically, the uterus was immersed in ammonium sulfate to aid visualization. Maternal organs of the cranial, thoracic and abdominal cavities and ovaries (including corpora lutea count) and uteri (implantation site count) were examined macroscopically. The uterine contents were classified as live fetuses, embryo/fetal deaths, placental remnants, early or late resorptions or macerated fetuses. Live fetuses were sexed, examined for external malformations and weighed.

There were no deaths and no clinical signs of toxicity at any dose level. At 1000 mg/kg bw per day, body weight gain from GD 6 was significantly reduced on GDs 7–8, whereas feed consumption was lower on GDs 6–11. There were no effects on water intake, the numbers of corpora lutea and implantations, the preimplantation and post-implantation loss rates, the number of live fetuses, sex ratio or live fetal body weights. Also, there were no external abnormalities of fetuses in any group.

The NOAEL for maternal toxicity was 300 mg/kg bw per day, based on reduced feed consumption and a transient decrease in body weight gain at 1000 mg/kg bw per day. The NOAEL for developmental toxicity was 1000 mg/kg bw per day, the highest dose tested (Sakurai, 1998a).

In a prenatal developmental toxicity study conducted according to OECD Test Guideline No. 414, groups of 24 mated female Crj:CD(SD) IGS (SPF) rats were administered dinotefuran (purity 92.9%) in 0.5% aqueous carboxymethylcellulose at a dose volume of 10 ml/kg bw by oral gavage at a dose level of 0, 100, 300 or 1000 mg/kg bw per day from GD 6 to GD 15. Mortality checks were performed and clinical signs recorded at least once daily on non-treatment days and at least twice daily during the treatment period. Body weights were recorded on GDs 0 and 3 and then daily from GD 5 until sacrifice. Feed consumption and water consumption were recorded on GDs 0 and 3 and then daily from GD 6 until sacrifice. Dams were sacrificed on GD 20 and the fetuses delivered by caesarean section. The uterine tract and ovaries were removed, and pregnancy was confirmed. If implantations were not visible macroscopically, the uterus was immersed in ammonium sulfate to aid visualization. Maternal organs of the cranial, thoracic and abdominal cavities and ovaries (including corpora lutea count) and uteri (implantation site count) were examined macroscopically. The uterine contents were classified as live fetuses, embryo/fetal deaths, placental remnants, early or late resorptions or macerated fetuses. Fetuses were sexed, examined for external malformations and weighed. Approximately one half of the fetuses (alternate) from each litter were examined for soft tissue malformations and variations by fixation in Bouin's solution and subsequent microdissection of the cranial and abdominal cavities by Wilson's method and of the thoracic cavity by the method of Nishimura. The remaining fetuses were subjected to skeletal evaluation using a dual staining technique for cartilage and bone and examined for skeletal malformations and variations, including counting the number of ossification centres in vertebrae, metacarpals, metatarsals, and proximal and medial phalanges.

There were no deaths during the study. With the exception of a single animal treated at 1000 mg/kg bw per day that showed transient hypoactivity on GDs 8–10, there were no clinical signs of toxicity at any dose level.

The body weight gain from day 6 to day 11 of the group treated at 1000 mg/kg bw per day was significantly reduced by 21%. Thereafter, weight gain was not significantly different from the controls, and on GD 20, the group mean body weights of all treated groups were not significantly different from control values (Table 42). The mean feed consumption of the group treated at 1000 mg/kg bw per day was significantly reduced by 10.5–13.0% on GDs 7, 8 and 10. The mean water consumption of this group was significantly increased by 19.8–23.8% on GDs 11–13. On other occasions during the treatment period, the feed consumption and water consumption at 1000 mg/kg bw per day were comparable to control values. There were no treatment-related effects on feed or water consumption in the groups treated at 100 or 300 mg/kg bw per day. There were no treatment-related macroscopic findings in the maternal animals at any dose level.

Table 42. Summary of selected maternal findings in the prenatal developmental toxicity study in rats

	0 mg/kg bw per day	100 mg/kg bw per day	300 mg/kg bw per day	1000 mg/kg bw per day
Body weight (g)				
- day 6	280	281	281	277
- day 20	386	390	390	373
Body weight gain (g)				
- days 6–11	21.9	22.0	22.2	17.3*
- days 6–15	41.2	40.3	43.0	37.3
- days 6–20	107	110	109	97
Feed consumption (g/day)				
- day 7	24.0	24.2	24.1	21.1**
- day 8	24.3	23.8	24.4	21.1**
- day 10	24.2	23.9	24.5	21.7*
Water intake (ml/day)				
- day 11	36.0	41.1	37.3	43.1*
- day 12	36.3	40.2	37.8	44.7*
- day 13	34.9	40.7	37.2	43.3*

From Sakurai (1998b)
* $P < 0.05$; ** $P < 0.01$

Litter parameters as assessed by pregnancy incidence, numbers of corpora lutea, implantations and live fetuses, post-implantation loss, external anomalies, fetal weights and sex ratio were unaffected by treatment at all dose levels. Although preimplantation loss in the group treated at 1000 mg/kg bw per day was high (24.0%) in relation to the control group (9.2%), it was not significantly different from the control value and is considered incidental to treatment with dinotefuran, as implantation was complete at the initiation of treatment. The mean number of implantations in the 1000 mg/kg bw per day group was slightly lower than, but not significantly different from, the control group value as a consequence of higher preimplantation loss (Table 43).

Table 43. Summary of selected reproductive data in the prenatal developmental toxicity study in rats

	0 mg/kg bw per day	100 mg/kg bw per day	300 mg/kg bw per day	1000 mg/kg bw per day
No. pregnant/ no. mated	22/24	22/24	20/24	20/24
No. of corpora lutea (mean/dam)	15.6	16.2	15.3	15.7
No. of implantations (mean/dam)	14.2	14.8	14.1	12.2
Preimplantation loss (%)	9.2	8.3	10.1	24.0
Total embryo/fetal loss (%)	5.1	5.1	3.4	3.6
Implant remnant (%)	0.0	0.0	0.0	0.0
Retained placenta (%)	4.3	3.7	3.4	2.7
Early death (%)	0.0	1.1	0.0	0.9
Late death (%)	0.8	0.0	0.0	0.0
Macerated fetuses (%)	0.0	0.3	0.0	0.0
No. of live fetuses (mean/dam)	13.5	14.0	13.6	11.8
Sex ratio (% males)	53.0	55.7	50.4	42.8
Mean body weight (g), males	3.73	3.72	3.83	3.71
Mean body weight (g), females	3.55	3.51	3.65	3.47
Total no. live fetuses	298	309	272	236
External abnormalities (no. fetuses)	0	0	0	0
Skeletal examination				
- no. of fetuses examined	143	150	132	114
- skeletal abnormalities (no. of fetuses/litters)	0/0	0/0	0/0	0/0
- skeletal variations (no. of fetuses/litters)	26/14	12/8	14/9	15/8
- skeletal variations (% of fetuses)	18.4	7.6	10.5	12.1
Visceral examination				
- no. of fetuses examined	155	159	140	122
- visceral abnormalities (no. of fetuses/litters)	8/6	4/3	6/5	5/5
- visceral abnormalities (% of fetuses)	5.0	3.5	8.4	5.1

From Sakurai (1998b)

There were no external fetal abnormalities in any of the treated or control groups. There were no treatment-related or statistically significant differences between treated and control groups in the incidence or nature of skeletal and visceral abnormalities and variations. No skeletal abnormalities occurred in any group, and the incidences of visceral abnormalities, thymic remnant, microphthalmia, ectopic ovary, pyeloectasia, ureteroectasia and left umbilical artery were similar in all groups. Delayed ossification, as assessed by the number of vertebral and phalangeal ossification centres, was not apparent at any dose level.

The NOAEL for maternal toxicity was 300 mg/kg bw per day, based on decreased weight gain and feed consumption and increased water consumption at 1000 mg/kg bw per day. The NOAEL for prenatal developmental toxicity was 1000 mg/kg bw per day, the highest dose tested (Sakurai, 1998b).

Rabbits

A single-dose study and a 2-week range-finding study were conducted in non-mated female New Zealand White rabbits in order to select the dose levels for the prenatal developmental toxicity study. Dinotefuran (purity 92.9%) in 0.5% aqueous carboxymethylcellulose was administered at a dose volume of 10 ml/kg bw by oral gavage to groups of two rabbits at a dose level of 0, 100, 300, 1000 or 2000 mg/kg bw in the single-dose study or to groups of three rabbits at a dose level of 0, 100, 300 or 1000 mg/kg bw per day in the 2-week study.

In the single-dose study, clinical signs (hypoactivity, sedation and flush of the nose and ears at 300 mg/kg bw, as well as panting, abdominal position, tremor, ptosis and side position at 1000 mg/kg bw and above) were observed on the day of dosing, whereas decreased feed consumption was observed at 1000 mg/kg bw and above from days 1 to 4 after dosing.

In the 2-week study, clinical signs were observed from the start of treatment and included hypoactivity, sedation, flush of the nose and ears and panting at 300 mg/kg bw per day, whereas at 1000 mg/kg bw per day, additionally abdominal position, tremor, ptosis, side position and bradypnoea were observed. Clinical signs ceased by day 6 of treatment, with the exception of hypoactivity at the top dose.

The NOAEL was 100 mg/kg bw per day in both the single-dose and 2-week studies, based on treatment-related clinical signs (hypoactivity, sedation, flush, panting) at 300 mg/kg bw per day and above (Sakurai, 1998c).

In a range-finding prenatal developmental toxicity study, groups of six mated female New Zealand White rabbits were administered dinotefuran (purity 92.9%) in 0.5% aqueous carboxymethylcellulose at a dose volume of 10 ml/kg bw by oral gavage at a dose level of 0, 100, 300 or 1000 mg/kg bw per day from GD 6 to GD 18. Clinical signs, body weight and feed consumption were recorded. On GD 28, dams were sacrificed and the fetuses delivered by caesarean section. The uterine tract and ovaries were removed, and pregnancy was confirmed. If implantations were not visible macroscopically, the uterus was immersed in 2% potassium hydroxide to aid visualization. Maternal organs of the cranial, thoracic and abdominal cavities and ovaries (including corpora lutea count) and uteri (implantation site count) were examined macroscopically. The uterine contents were classified as live fetuses, embryo/fetal deaths, placental remnants, early or late resorptions or macerated fetuses. Live fetuses were sexed, examined for external malformations and weighed.

There were no deaths at any dose level. Abortion occurred in four dams at 1000 mg/kg bw per day and in one control dam, and one control dam was non-pregnant. At 1000 mg/kg bw per day, dams showed hypoactivity, flush of auricle and nose, panting, prone position, tremors and sedation on GDs 6–9, whereas only hypoactivity was observed from GD 10 to GD 18. At 300 mg/kg bw per day, dams showed hypoactivity and panting on GDs 6–9, and two dams showed tremors on GD 8 or 9. There were no clinical signs at 100 mg/kg bw per day. Decreases in body weight gain and feed consumption were observed at 1000 mg/kg bw per day, whereas water intake was not affected. At necropsy, grey-white plaques in the fundic region of the stomach were seen in dams at all dose levels, whereas pale brown discoloration of the liver was observed in one, three or four dams of the low-, mid- and high-dose groups, respectively. There were no significant differences in the numbers of corpora lutea and implantations, the preimplantation and post-implantation loss rates, the number of live fetuses, sex ratio or live fetal body weights. Also, there were no treatment-related external abnormalities of fetuses in any group.

The NOAEL for maternal toxicity was 100 mg/kg bw per day, based on treatment-related clinical signs (hypoactivity, panting, tremor) at 300 mg/kg bw per day and above. The NOAEL for developmental toxicity was 1000 mg/kg bw per day, the highest dose tested (Sakurai, 1998d).

In a prenatal developmental toxicity study conducted according to OECD Test Guideline No. 414, groups of 22 mated female New Zealand White rabbits were administered dinotefuran (purity

92.9%) in 0.5% aqueous carboxymethylcellulose at a dose volume of 10 ml/kg bw by oral gavage at a dose level of 0, 52, 125 or 300 mg/kg bw per day from GD 6 to GD 18. Mortality checks were performed and clinical signs recorded at least once daily on non-treatment days and at least twice daily during the treatment period. Body weights were recorded on GD 0, daily from GD 6 to GD 19, on GDs 21, 23, 25 and 27 and on the day of necropsy. Feed consumption and water consumption were recorded daily from GD 6 until necropsy. Dams were sacrificed on GD 28 and the fetuses delivered by caesarean section. The uterine tract and ovaries were removed, and pregnancy was confirmed. If implantations were not visible macroscopically, the uterus was immersed in 2% potassium hydroxide to aid visualization. Maternal organs of the cranial, thoracic and abdominal cavities and ovaries (including corpora lutea count) and uteri (implantation site count) were examined macroscopically. Gross lesions were preserved for subsequent histological examination. The uterine contents were classified as live or dead fetuses, placental remnants, early or late resorptions or macerated fetuses. Live fetuses were weighed and examined for external and oral cavity abnormalities. The thoracic and abdominal organs were examined macroscopically, and sexes were recorded by examination of the internal reproductive organs. The heads of approximately one half of the fetuses from each litter and the thoracic viscera from all fetuses were examined for soft tissue malformations and variations by fixation in Bouin’s solution and subsequent microdissection of the cranial cavities and of the thoracic cavity. The carcasses of all fetuses were subjected to skeletal evaluation using a dual staining technique for cartilage and bone and examined for skeletal malformations and variations, including enumeration of ossification centres in vertebrae, metacarpals, metatarsals, and right limb proximal and medial phalanges.

There were no treatment-related deaths or abortions during the study, but a control animal died on GD 7 due to incorrect dosing. One animal to be treated at 300 mg/kg bw per day was excluded from the study on GD 6 due to body weight loss prior to the start of treatment. Clinical signs of toxicity were confined to the group treated at 300 mg/kg bw per day. This group showed hypoactivity, prone position, panting, erythema of the nose and ears and tremor from the start of treatment until GD 13.

The mean body weight gain of the group treated at 300 mg/kg bw per day was significantly reduced by 50% during the treatment period (Table 44). After that, the group gained weight at a greater rate than the controls, and body weights were similar to those of the control group at termination. The mean body weight gain of the group treated at 125 mg/kg bw per day was slightly, but significantly, reduced on GD 8 only. The mean weight gain of the group treated at 52 mg/kg bw per day was unaffected by treatment with dinotefuran. The mean feed consumption during treatment of the group treated at 300 mg/kg bw per day was reduced by 22.7%, and water consumption was significantly reduced by up to 34.2% on GDs 14–16. The feed consumption and water consumption of the groups treated at 125 or 52 mg/kg bw per day were unaffected by treatment with dinotefuran.

Treatment-related macroscopic findings in maternal animals occurred at 125 and 300 mg/kg bw per day (Table 44). Pale brown discoloration of the liver and grey-white plaque formation in the fundic region of the stomach occurred in most animals treated at 300 mg/kg bw per day and in a smaller proportion of animals treated at 125 mg/kg bw per day. One animal at 300 mg/kg bw per day also showed liver enlargement. Histological examination of representative maternal liver and stomach lesions revealed no correlative histopathological alterations. No macroscopic changes occurred at 52 mg/kg bw per day.

There were no treatment-related effects on pregnancy incidence, numbers of corpora lutea, implantations and live fetuses, post-implantation loss, external anomalies, fetal weights or sex ratio at all dose levels (Table 45).

There were no treatment-related effects on the incidence or nature of skeletal and visceral abnormalities and variants at any dose level. The incidences of skeletal and visceral abnormalities and skeletal variants were not significantly different from those of the control group. Although one litter from a dam treated at 300 mg/kg bw per day had three fetuses with hydrocephalus, this abnormality occurs spontaneously in rabbits of the strain and source employed. Delayed ossification, measured by the number of vertebral and phalangeal ossification centres, was not apparent at any dose level.

Table 44. Summary of selected maternal findings in the prenatal developmental toxicity study in rabbits

	0 mg/kg bw per day	52 mg/kg bw per day	125 mg/kg bw per day	300 mg/kg bw per day
No. of animals mated	22	22	22	22
No. of animals died or killed in extremis	1	0	0	1
No. of animals with clinical signs of toxicity	0	0	0	21
Body weight (kg)				
- day 6	3.31	3.27	3.42	3.37
- day 8	3.33	3.28	3.39	3.33
- day 19	3.53	3.51	3.61	3.48
- day 28	3.71	3.67	3.78	3.73
Body weight gain (g)				
- days 6–8	0.017	0.016	−0.037*	−0.039*
- days 6–10	0.050	0.046	−0.006	−0.009
- days 6–14	0.145	0.182	0.105	0.019**
- days 6–19	0.224	0.246	0.183	0.107*
Feed consumption (g/day)				
- day 6	155	154	159	152
- day 8	154	161	138	118*
- day 14	153	162	157	77**
- day 16	152	156	156	107**
Water intake (ml/day)				
- day 7	439	401	411	399
- day 14	482	506	488	317*
- day 16	503	505	507	380*
No. of animals examined at necropsy	22	22	22	22
Pale brown discoloration of liver	0	0	8	20
Liver enlargement	0	0	0	1
Grey-white discoloration of gastric mucosa	0	0	0	1
Grey-white plaque in fundus of stomach	0	0	15	20
Thickening of gastric mucosa	0	0	0	2

From Sakurai (1998e)
* $P < 0.05$; ** $P < 0.01$

The NOAEL for maternal toxicity was 52 mg/kg bw per day, based on a reduction in body weight gain at 125 mg/kg bw per day and above. The NOAEL for acute clinical signs in dams was 125 mg/kg bw per day, based on the manifestation of hypoactivity, prone position, panting, erythema and tremor from the start of treatment until GD 13. The NOAEL for developmental toxicity was 300 mg/kg bw per day, the highest dose tested (Sakurai, 1998e).

Table 45. Summary of selected reproductive data in the prenatal developmental toxicity study in rabbits

	0 mg/kg bw per day	52 mg/kg bw per day	125 mg/kg bw per day	300 mg/kg bw per day
No. mated / no. pregnant	22 / 20	22 / 19	22 / 21	22 / 19
No. of corpora lutea (mean/dam)	8.6	8.5	9.0	8.8
No. of implantations (mean/dam)	7.9	8.3	8.7	8.4
Preimplantation loss (%)	7.7	3.2	5.0	4.4
Total embryo/fetal loss (%)	5.1	2.1	6.9	6.9
Implant remnant (%)	0	0	0	0
Retained placenta (%)	4.1	2.1	2.9	1.8
Early death (%)	0	0	1.3	0.5
Late death (%)	0.5	0	2.1	3.6
Macerated fetuses (%)	0.5	0	0.6	1.0
No. of live fetuses (mean/dam)	7.6	8.1	8.1	7.7
Sex ratio (% males)	59.2	43.9	52.0	51.6
Mean body weight (g), males	42.7	40.6	39.7	40.4
Mean body weight (g), females	42.1	39.9	38.7	39.9
Total no. of live fetuses	151	153	170	147
External abnormalities (no. of fetuses/litters)	0/0	1/1	2/2	1/1
Skeletal examination				
- no. of fetuses examined	151	153	170	147
- skeletal abnormalities (no. of fetuses/litters)	2/2	0/0	1/1	0/0
- skeletal variations (no. of fetuses/litters)	92/20	106/19	126/21	101/19
- skeletal variations (% of fetuses)	58.9	70.2	60.7	67.6
Visceral examination				
- no. of fetuses examined	151	153	170	138
- visceral abnormalities (no. of fetuses/litters)	2/2	1/1	3/3	4/2
- visceral abnormalities (% of fetuses)	1.6	0.7	2.9	3.0

From Sakurai (1998e)

2.6 Special studies

(a) Neurotoxicity

In an acute neurotoxicity study conducted in accordance with OECD Test Guideline No. 424, groups of 10 male and 10 female Crl:CD®(SD) IGS BR rats received dinotefuran (purity 93.0%) in 0.5% aqueous carboxymethylcellulose at a dose volume of 20 ml/kg bw by oral gavage at a single dose of 0, 325, 750 or 1500 mg/kg bw and were then maintained for a 14-day observation period. The animals were observed twice daily for morbidity/mortality and daily for clinical signs. Body weights were recorded predosing and on days 1, 8 and 15, and individual feed consumption was measured weekly. All animals were subjected to a functional observational battery predosing, 3 hours after treatment on day 1 (the estimated time of peak effect) and on days 8 and 15. The tests and observations were performed without knowledge of the treatment of each animal. The functional observational battery comprised a series of qualitative and semiquantitative observations made in the home cage, during handling, in an open arena and during manipulations to assess reflex responses and physiological parameters. The assessments included evaluation of posture, activity, gait, locomotor

activity, unusual behaviour, reactivity to handling, vocalization, palpebral closure, exophthalmos, lacrimation, salivation, respiration, appearance of fur, piloerection, muscle tone, pupillary status, latency to first step in an open field, grooming and rearing activity, defecation, micturition, auditory reactivity, proprioceptive positioning, pinna response, approach response, righting reflex, corneal touch response, nociceptive reflexes and hindlimb foot splay. Quantitative measurements were made of rectal temperature, forelimb and hindlimb grip strength, and motor activity counts for 2-minute intervals for 40 minutes.

During week 3, the animals were deprived of feed overnight and subjected to necropsy and postmortem examination of major organs and tissues. The tissues of all animals were perfusion-fixed in situ, and six animals of each sex from the groups treated at 0 or 1500 mg/kg bw that were suitably perfusion-fixed were designated for neuropathological evaluation. The brain, entire spinal cord, pituitary, cervical and lumbar dorsal root ganglia, trigeminal ganglia, eyes, fibular, optic, sciatic, tibial and sural nerves, anterior tibialis and gastrocnemius muscles and gross lesions were preserved from all animals. The olfactory bulb, forebrain, caudate nucleus, hypothalamus/thalamus, midbrain, cerebellum, medulla, pituitary, three levels of spinal cord, eye, skeletal muscles and gross lesions from the six animals of each sex in the groups treated at 0 or 1500 mg/kg bw were processed to paraffin blocks, and sections were stained with haematoxylin and eosin. Dorsal root and trigeminal ganglia and all peripheral nerves from the same animals were embedded in epoxy resin, sectioned and stained with toluidine blue. The stained sections were examined microscopically.

There were no deaths or treatment-related clinical signs during the study at any dose level. There were no effects on body weight gain or feed consumption at any dose level. The group mean body weights and feed consumption of each treated group of each sex varied by less than 5% and 6%, respectively, from the controls. There were some minor variations in the functional observational battery observations, but none was considered to be treatment related, because they were not dose related, occurred also during the predosing evaluation or occurred at very low incidence, or there were no other correlating behavioural changes. Therefore, there were no treatment-related effects in either sex at any dose level on the qualitative and semiquantitative evaluation of functional observational battery observations, reflexes and responses at any of the testing intervals. The motor activity of both sexes at 1500 mg/kg bw, as measured quantitatively, was reduced on day 1 relative to predosing values and to the controls. The effect was statistically significant in females for the first 10-minute interval, the last 10-minute interval and the total 40-minute interval, but in males for the first 10-minute interval only. The effect did not occur subsequently at 1500 mg/kg bw. Motor activity at lower dose levels was unaffected by treatment at all testing intervals (Table 46).

Table 46. Summary of motor activity findings in the acute neurotoxicity study in rats

Time point	Motor activity (activity counts)							
	Males				Females			
	0 mg/kg bw	325 mg/kg bw	750 mg/kg bw	1500 mg/kg bw	0 mg/kg bw	325 mg/kg bw	750 mg/kg bw	1500 mg/kg bw
Pre-exposure	1369	1365	1377	1323	1147	1016	1061	1151
Day 1	809	1074	837	567[#]	1006	940	682	531*
Day 8	1302	1529	1471	1435	1400	1105	1118	1175
Day 15	1389	1662	1650	1363	1375	1258	1157	1068

From Weiler (2001a)

* $P < 0.05$; [#] $P < 0.05$ for the first 10-minute interval only

There were no other treatment-related effects in either sex at any dose level on the quantitative evaluation of grooming and rearing activity, defecation, micturition, grip strength, nociceptive reflex, foot splay or rectal temperature.

Macroscopic examination at necropsy revealed no treatment-related lesions at any dose level. There were few histopathological findings in the central and peripheral nervous tissues and other tissues examined, and the nature and distribution between the groups did not indicate an effect of treatment at 1500 mg/kg bw. All histopathological findings were considered incidental to treatment and common to animals of the strain and age used.

The NOAEL in this study was 750 mg/kg bw, based on a transient decrease in motor activity at 1500 mg/kg bw. The NOAEL for neuropathological effects was 1500 mg/kg bw, the highest dose tested (Weiler, 2001a).

In a subchronic neurotoxicity study conducted in accordance with OECD Test Guideline No. 424, groups of 10 male and 10 female Crl:CD®(SD) IGS BR rats were fed diets containing dinotefuran (purity 93.0%) at a concentration of 0, 500, 5000 or 50 000 ppm (equal to 0, 33, 327 and 3413 mg/kg bw per day for males and 0, 40, 400 and 3806 mg/kg bw per day for females, respectively) for at least 13 weeks. The animals were observed twice daily for morbidity/mortality and daily for clinical signs. Body weights were recorded predosing, on day 1 and weekly thereafter. Body weights were also recorded on the days of functional observational battery testing. Individual feed consumption was measured weekly. All animals were subjected to a battery of behavioural tests and observations (functional observational battery) predosing and during weeks 2, 4, 8 and 13. The tests and observations were performed without knowledge of the treatment of each animal. The functional observational battery comprised a series of qualitative and semiquantitative observations made in the home cage, during handling, in an open arena and during manipulations to assess reflex responses and physiological parameters. The assessments included evaluation of posture, activity, gait, locomotor activity, unusual behaviour, reactivity to handling, vocalization, palpebral closure, exophthalmos, lacrimation, salivation, respiration, appearance of fur, piloerection, muscle tone, pupillary status, latency to first step in an open field, grooming and rearing activity, defecation, micturition, auditory reactivity, proprioceptive positioning, pinna response, approach response, righting reflex, corneal touch response, nociceptive reflexes and hindlimb foot splay. Quantitative measurements were made of rectal temperature, forelimb and hindlimb grip strength, and motor activity counts for 2-minute intervals for 40 minutes. During week 14, the animals were deprived of feed overnight and subjected to necropsy and postmortem examination of major organs and tissues. The tissues of all animals were perfusion-fixed in situ, and six animals of each sex from the groups treated at 0 or 50 000 ppm that were suitably perfusion-fixed were designated for neuropathological evaluation. The brain, entire spinal cord, pituitary, cervical and lumbar dorsal root ganglia, trigeminal ganglia, eyes, fibular, optic, sciatic, tibial and sural nerves, anterior tibialis and gastrocnemius muscles and gross lesions were preserved from all animals. The olfactory bulb, forebrain, caudate nucleus, hypothalamus/thalamus, midbrain, cerebellum, medulla, pituitary, three levels of spinal cord, eye, skeletal muscles and gross lesions from the six animals of each sex in the groups treated at 0 or 50 000 ppm were processed to paraffin blocks, and sections were stained with haematoxylin and eosin. Dorsal root and trigeminal ganglia and all peripheral nerves from the same animals were embedded in epoxy resin, sectioned and stained with toluidine blue. The stained sections were examined microscopically.

There were no deaths during the study and no treatment-related clinical signs at any dose level, but the overall body weight gain of both sexes treated at 50 000 ppm was significantly reduced. The group mean body weights at 50 000 ppm were significantly lower than those of the controls from week 2 of the study and at termination were 20.8% and 18.9% lower than control values in males and females, respectively. The effect on body weight at 50 000 ppm was accompanied by reduced feed consumption, which was frequently significantly lower than control values. The body weight gain and feed consumption of both sexes at lower dose levels were unaffected by treatment with dinotefuran.

There were no treatment-related effects in either sex at any dose level at any of the assessment intervals on the qualitative and semiquantitative observations made in the home cage, during handling, in an open arena and during manipulations to assess reflex responses and physiological parameters. Similarly, there were no treatment-related effects in either sex at any dose level at any of the assessment intervals on the quantitative parameters of grip strength, speed of

nociceptive reflex, foot splay and body temperature. Although minor statistically significant differences between the groups were apparent for rearing activity (50 000 ppm females in week 2), number of urine pools (5000 and 50 000 ppm males in week 4), hindlimb grip strength (500 ppm females in week 2), foot splay (50 000 ppm females in week 8) and body temperature (50 000 ppm females in week 2), none was considered to be treatment related, because they were not dose related, there was no consistency between testing intervals or the numerical differences were small. Motor activity in females at 50 000 ppm was significantly lower than that of the controls in week 2 during each 10-minute interval and during the entire 40-minute test period (Table 47). As a reduction in motor activity was also observed in the acute neurotoxicity study, a relationship to treatment was considered plausible. All other quantitative assessments in dinotefuran-treated groups were comparable to, and not significantly different from, control values.

Table 47. Summary of selected findings in the subchronic neurotoxicity study in rats

	Males				Females			
	0 ppm	500 ppm	5000 ppm	50 000 ppm	0 ppm	500 ppm	5000 ppm	50 000 ppm
Body weight (g)								
- week 1	256	251	257	249	174	183	173	180
- week 2	295	292	298	245*	197	199	189	165*
- week 4	370	358	363	300*	226	227	218	193*
- week 8	461	454	450	365*	257	262	251	217*
- week 14	514	511	508	407*	286	291	277	232*
Body weight gain (g), weeks 1–14	258	260	251	159*	112	107	103	52*
Feed consumption (g/week)								
- week 1	174	177	182	132*	138	141	124	87*
- week 2	182	176	181	156*	131	134	125	114*
- week 4	191	184	185	174	132	138	130	107*
- week 8	182	187	183	173	128	137	128	107*
- week 13	175	186	183	156	126	134	137	99*
Motor activity (activity counts)								
- pre-exposure	1115	1219	1098	1231	857	1177	1194	923
- week 2	1179	1437	1643	1050	1280	1346	1742	705*
- week 4	1321	1561	1686	1394	1462	1358	1577	1049
- week 8	1532	1462	1902	1630	1386	1502	1604	1165
- week 13	1298	1436	1559	1789	1585	1678	1648	1234

From Weiler (2001b)
* $P < 0.05$

There were no treatment-related gross lesions at necropsy at any dose level and no treatment-related microscopic lesions in the animals treated at 50 000 ppm in central and peripheral nervous tissues, skeletal muscle and other tissues examined. Histopathological alterations were infrequent and generally occurred in isolation, and the distribution between the control and dinotefuran-treated group did not indicate an effect of treatment.

The NOAEL for neurotoxicity in male rats was 50 000 ppm (equal to 3413 mg/kg bw per day), the highest dose tested. In female rats, a transient decrease in motor activity was observed at 50 000 ppm (equal to 3806 mg/kg bw per day), which is well above the limit dose. The NOAEL for systemic toxicity was 5000 ppm (equal to 327 mg/kg bw per day for males and 400 mg/kg bw per day

for females), based on reduced body weight gain and feed consumption at 50 000 ppm (Weiler, 2001b).

(b) Developmental neurotoxicity and immunotoxicity

In a dose range–finding developmental neurotoxicity and immunotoxicity study, groups of 10 mated female Crl:CD(SD) rats were given diets containing dinotefuran (purity 95.6%) at a concentration of 0, 1000, 3000 or 10 000 ppm from GD 6 through lactation day (LD) 21. F_1 generation rats were treated continuously after weaning until sacrifice. The mean test substance intake for the P generation females at 1000, 3000 and 10 000 ppm was equal to 69.5, 212 and 670 mg/kg bw per day during gestation and 141, 424 and 1401 mg/kg bw per day during lactation, respectively. For the F_1 generation, the mean test substance intake at 1000, 3000 and 10 000 ppm was equal to 100, 311 and 1043 mg/kg bw per day for males and 112, 316 and 1120 mg/kg bw per day for females, respectively. In P generation females, the day on which a vaginal plug, or sperm in a smear, was detected was designated day 0 of gestation (GD 0); the day of birth was designated postnatal day 0 (PND 0)/day 0 of lactation (LD 0).

P generation females were observed twice daily for viability and for clinical signs and general appearance weekly at approximately the same time each week during the pre-exposure period and on GD 0. The dams were also examined for clinical observations, abortions, premature deliveries and deaths on GDs 6, 9, 12, 15, 18, 20 and 25 (rats that did not deliver a litter) and LDs 0, 4, 7, 13 and 21. Body weights were recorded weekly during the pre-exposure period and on GDs 0, 6, 9, 12, 15, 18, 20 and 25 (if necessary) and LDs 0, 4, 7, 13 and 21. Feed consumption was recorded on GDs 0, 6, 9, 12, 15, 18, 20 and 25 (if necessary) and LDs 0, 4, 7 and 13. Rats were evaluated for adverse clinical signs observed during parturition, duration of gestation, litter sizes, live litter size and pup viability at birth. Maternal behaviour was evaluated on LDs 0, 4, 7, 13 and 21, and variations from expected maternal behaviour were recorded on all other days of the postpartum period. On LD 21, dams were sacrificed, and a gross necropsy of thoracic, abdominal and pelvic viscera was performed. The number and distribution of implantation sites were recorded. Rats that did not deliver a litter were sacrificed on GD 25 and examined for gross lesions. Uteri were examined to confirm the absence of implantation sites.

All pups in a litter were individually weighed on the day of birth and on PNDs 4, 7, 11, 13, 17 and 21. Each litter was evaluated for viability at least twice daily. The number of pups in each litter and clinical observations were recorded once daily during the preweaning period. Body weights were recorded weekly during the post-weaning period and prior to sacrifice. Feed consumption was measured weekly during the post-weaning period. On PND 4, the litter size was reduced, by random selection, to six pups of each sex, when possible. On PND 21, 20 pups of each sex per group (up to 3 pups of each sex per litter) were selected randomly for each of the two immunological assays, when possible. The first one or two pups of each sex per litter were selected for assay 1, and the last one or two pups of each sex per litter were assigned to assay 2.

Pups that died before initial examination of the litter for pup viability were evaluated for vital status at birth. The lungs were removed and immersed in water. Pups with lungs that sank were considered stillborn; pups with lungs that floated were considered liveborn and to have died shortly after birth. Pups found dead were examined for gross lesions and for the cause of death. On PND 4, all offspring selected for standardization were sacrificed and necropsied. On PND 21, all F_1 generation pups not selected for assays 1 and 2 were sacrificed and examined for gross lesions.

In immunological assay 1, rats were sacrificed 4 days following sensitization with the injection of sheep red blood cells and completion of the exposure period (PNDs 36–40 for male rats and PNDs 43–47 for female rats). Rats were examined for gross lesions, and spleens were harvested aseptically. The primary immunoglobulin M (IgM) response to sheep red blood cells was measured using a modified haemolytic plaque assay. Spleen cell number, following lysis of red blood cells, was determined. The cells per spleen, antibody-forming cells (AFC) per 10^6 spleen cells (specific activity), AFC/spleen (total spleen activity) and plaques were determined.

In immunological assay 2, rats were sacrificed following completion of the exposure period (PNDs 36–42 for male rats and PNDs 45–49 for female rats). Rats were examined for gross lesions, and spleens were harvested aseptically. The spleen cell phenotypes were evaluated using the following antibodies: CD45RA for total B cells, CD5 for total T cells, CD4 for T helper cells and CD8 for cytotoxic T cells. Natural killer (NK) cells were enumerated as cells that were NKP-P1A positive and CD8a negative. The samples were processed by flow cytometry for evaluation and enumeration. In the NK cell assay, the NK cell was used as the effector cell, and mouse lymphoma YAC-1 cells, introduced by inoculation of the Moloney leukaemia virus into a newborn A/Sn mouse, was used as the target cell. The mean per cent cytotoxicity at each effector concentration was determined.

All P generation female rats survived until scheduled sacrifice. There were no treatment-related clinical signs or gross lesions at necropsy. Body weights and body weight gains during the gestation and lactation periods were unaffected by exposure to the test substance at all dose levels. For absolute and relative feed consumption, all values were comparable among the dose groups and did not significantly differ, with the exception of absolute and relative feed consumption values for GDs 6–9, which were significantly reduced (about 10%) in the 10 000 ppm group (Table 48). Pregnancy occurred in 9, 9, 10 and 10 of the 10 mated female rats in the 0, 1000, 3000 and 10 000 ppm groups, respectively. All pregnant dams delivered litters. There were no effects of treatment on the numbers of dams delivering litters, the duration of gestation, the numbers of implantation sites per litter, the gestation index, the numbers of dams with stillborn pups and of dams with all pups dying, litter sizes, viability and lactation indices, surviving pups per litter, sex ratio or live litter size at weighing.

Pup weights in the 10 000 ppm group were significantly reduced on PNDs 13, 17 and 21. These reductions were probably due to direct exposure of the pups to the test substance in the diet as well as exposure through the milk, as pups start to eat feed at approximately PND 13. No deaths and no treatment-related clinical signs or gross lesions at necropsy were observed at any dose level.

In F_1 pups designated for the immunological assays, there were no treatment-related clinical signs, deaths or gross lesions at any dose level. However, one female rat in the 1000 ppm exposure group was found dead on PND 25, but death was considered not to be treatment related. Body weights of male and female rats were significantly lower than control values in the 1000 ppm and higher exposure groups on PND 22. However, significantly lower body weights continued only in the 10 000 ppm exposure group, through PND 57 for male rats and for PNDs 29, 36, 57 and 64 for female rats (Table 49). Significantly lower body weights in male rats occurred in the 1000 ppm exposure group on PND 29, but this reduction was not dose dependent and not considered related to the test substance. Body weight gains of male and female rats were unaffected by exposures to the test substance at all dose levels. No significant differences occurred among the groups with the exception of PNDs 57–64 in the 3000 and 10 000 ppm female exposure groups, where a non-dose-dependent but statistically significant reduction occurred. The general lack of statistically significant reductions in body weight gains indicates that the lower body weights that occurred on PND 22 were due to the high dose of test substance consumed at or near weaning. Feed consumption during the post-weaning period was unaffected by exposure to the test substance at concentrations as high as 10 000 ppm.

In immunological assay 1, exposure to dinotefuran did not produce dose-dependent decreases in the IgM AFC responses to sheep red blood cells when evaluated either as specific activity or as total spleen activity for both male and female rats (Table 49).

In immunological assay 2, terminal body weights in male rats and spleen weights (absolute and relative) in male and female rats did not differ significantly among the groups compared with the control group values. Terminal body weights in female rats were significantly reduced at 3000 and 10 000 ppm. Overall, the phenotypic analysis of splenic subpopulations demonstrated a lack of effect at all dose levels on the numbers of total B cells ($CD45^+$), total T cells ($CD5^+$), helper/delayed-type sensitivity (DTH) T cells ($CD4^+CD5^+$), cytotoxic T cells ($CD8^+CD5^+$) and NK cells (NKR-PIA^+CD8^-). Although males at 10 000 ppm showed a significantly lower group mean absolute number of NK cells, this was due to a lower absolute number of spleen cells, as the relative (per cent)

value for NK cells was comparable to the control value. In the NK cell assay, there was no effect on NK cell activity after exposures as high as 10 000 ppm in either male or female rats (Table 49).

Table 48. Selected maternal and litter data in the dose range–finding developmental neurotoxicity and immunotoxicity study in rats

	0 ppm	1000 ppm	3000 ppm	10 000 ppm
Maternal body weight (g)				
- GD 0	229	229	230	230
- GD 9	270	271	276	270
- GD 20	362	366	378	363
- LD 0	279	279	289	274
- LD 21	292	287	296	289
Maternal feed consumption (g/day)				
- GDs 0–6	18.6	19.0	19.7	19.2
- GDs 6–9	21.2	21.0	21.2	19.2**
- GDs 9–12	21.4	21.8	21.8	20.7
- GDs 15–18	21.8	21.7	23.4	21.9
Litters with liveborn pups/no. of pups delivered	9/118	9/126	10/139	10/139
Live litter size				
- PND 0	12.9	14.0	13.9	13.9
- PND 21	11.4	12.0	11.9	12.0
Pup weight/litter (g)				
- PND 0	6.7	6.2	6.5	6.6
- PND 4, pre-cull	10.6	9.9	10.5	10.3
- PND 4, post-cull	10.7	10.0	10.6	10.4
- PND 7	15.8	14.9	15.6	15.1
- PND 11	22.6	21.1	21.3	20.7
- PND 13	26.0	23.9	23.9	22.6**
- PND 17	33.0	30.0*	30.6	28.1**
- PND 21	46.7	41.5*	42.3	38.2**

From Hoberman (2009)
* $P < 0.05$; ** $P < 0.01$

The NOAEL for maternal toxicity was 10 000 ppm (equal to 670 mg/kg bw per day), the highest dose tested. The NOAEL for toxicity in offspring was 3000 ppm (equal to 311 mg/kg bw per day for males and 316 mg/kg bw per day for females), based on reduced body weight and body weight gain at 10 000 ppm. The NOAEL for developmental neurotoxicity and immunotoxicity was 10 000 ppm (equal to 1043 mg/kg bw per day for males and 1120 mg/kg bw per day for females), the highest dose tested (Hoberman, 2009).

In a developmental neurotoxicity study conducted in accordance with OECD Test Guideline No. 426, groups of 25 bred female Crl:CD(SD) rats were given diets containing dinotefuran (purity 99.5%) at a concentration of 0, 1000, 3000 or 10 000 ppm from GD 6 through LD 21. The mean test substance intakes at 1000, 3000 and 10 000 ppm were equal to 79.4, 237 and 784 mg/kg bw per day through gestation and 158, 501 and 1643 mg/kg bw per day through lactation, respectively. The day on which a vaginal plug, or sperm in a smear, was detected was designated GD 0; the day of birth was

designated LD 0 or PND 0. Direct dosing of F_1 generation pups during the lactation period was not performed, as the transfer of dinotefuran into the milk of lactating females has been confirmed (Hassler, 2006). F_1 generation offspring selected for study beyond PND 21 were not treated.

Table 49. Selected F_1 generation data in the dose range–finding developmental neurotoxicity and immunotoxicity study in rats

	Males				Females			
	0 ppm	1000 ppm	3000 ppm	10 000 ppm	0 ppm	1000 ppm	3000 ppm	10 000 ppm
Body weight (g)								
- PND 22	53.4	47.7**	48.8*	41.8**	51.8	44.4**	46.8*	42.1**
- PND 29	96.3	90.0*	93.6	83.3**	88.9	82.8	86.2	79.2**
- PND 36	152.4	146.2	151.6	135.8**	131.7	124.4	128.4	119.6**
- PND 43	214.0	204.8	212.1	193.4**	163.8	156.7	159.2	151.6
- PND 50	274.0	266.7	269.6	250.5**	189.4	184.8	184.6	176.1
- PND 57	332.0	327.1	330.0	308.1**	214.2	206.5	208.8	198.6*
- PND 64	—	—	—	—	235.8	225.4	223.4	214.3**
Body weight change (g)								
- PNDs 22–29	42.8	42.3	44.8	41.5	37.2	37.6	39.3	37.0
- PNDs 29–36	56.2	56.2	58.0	52.5	42.8	41.6	42.2	40.4
- PNDs 36–43	61.6	58.6	60.5	57.6	32.0	32.3	30.8	32.0
- PNDs 43–50	59.9	62.0	57.4	57.0	25.7	28.1	25.4	24.6
- PNDs 50–57	58.1	60.4	60.4	57.6	24.8	21.7	24.2	22.4
- PNDs 57–64	—	—	—	—	21.6	18.9	14.6**	15.8**
- PNDs 22–57	278.6	279.4	281.2	266.3	—	—	—	—
- PNDs 22–64	—	—	—	—	184.0	180.3	176.6	172.2
Immunological assay 1								
Body weight (g)	344	354	356	340	239	238	234	230
Spleen weight (mg)	619	846**	803*	776	521	546	549	557
Spleen cells ($\times 10^7$)	78.5	82.8	102	85.4	61.8	60.1	61.9	59.0
IgM AFC/10^6 spleen cells	1172	670	1027	909	2198	1673	1496	1668
IgM AFC/spleen ($\times 10^3$)	912	611	1022	788	1400	960	918	929
Immunological assay 2								
Body weight (g)	369	370	374	341	254	246	235*	218**
Spleen weight (mg)	749	774	733	671	498	512	476	482
Spleen cells ($\times 10^7$)	88.0	88.6	74.4	69.7*	57.3	62.8	59.6	55.7
Total B cells	485.2	503.2	433.6	386.3	310.6	323.8	348.5	316.0
Total T cells	251.4	246.0	195.9	209.5	167.6	203.7	162.9	163.1
Helper/DTH T cells	139.9	131.0	112.2	124.5	99.4	117.9	97.0	92.3
Cytotoxic T cells	109.8	111.2	83.3	83.8	74.8	90.8	68.8	72.8
NK cells	140.3	142.4	110.9	109.8*	97.8	114.6	90.7	95.8

From Hoberman (2009)

AFC, antibody-forming cell; DTH, delayed-type hypersensitivity; IgM, immunoglobulin M; NK, natural killer; * $P < 0.05$; ** $P < 0.01$

Parental generation females were observed twice daily for viability and for clinical signs and general appearance weekly at approximately the same time each week during the pre-exposure period and on GD 0. The dams were also examined for detailed clinical observations daily starting on GD 6 by an observer unaware of the dose groups. Body weights were recorded weekly during the pre-exposure period, on GD 0 and daily thereafter. Feed consumption was recorded on GD 0 and daily during all other periods. Dams were evaluated for adverse clinical signs observed during parturition, duration of gestation, litter sizes, live litter size and pup viability at birth. Maternal behaviour was evaluated on LDs 0, 4, 7, 13 and 21. Litters were standardized to five pups of each sex (where possible) on LD 4, and litters of fewer than nine pups were not assigned to study, but retained until needed. Parental generation females delivering a litter were sacrificed, and a gross necropsy of thoracic, abdominal and pelvic viscera was performed. The number and distribution of implantation sites were recorded. Female rats that did not deliver a litter were sacrificed on presumed GD 25 and examined for gross lesions. Uteri were examined to confirm the absence of implantation sites.

All pups in a litter were individually weighed on PNDs 0, 4, 7, 11, 13, 17 and 21. Each litter was evaluated for viability at least twice daily. The number of pups in each litter and clinical observations were recorded once daily during the preweaning period. Offspring were observed for viability at least twice daily, and clinical observations were recorded daily during the preweaning period. Body weights were recorded on PNDs 0, 4, 7, 11, 13, 17 and 21 and weekly during the post-weaning period and prior to sacrifice for pups selected for further study (subsets 1–5). Feed consumption was measured weekly during the post-weaning period. Pups that died before scheduled termination and not selected for further study were examined for gross lesions and the cause of death as soon as possible. Pups found dead on days 1–4 were preserved for possible future evaluation. All offspring not selected for continued evaluation were necropsied prior to weaning, and gross lesions were subjected to histological evaluation. Further evaluations were performed on the following subsets using, where possible, one pup of each sex per litter:

- *Subset 1 (up to 25 rats of each sex per group)*: Ten rats of each sex per group were sacrificed on PND 21 for recording of brain weight/gross dimensions and microscopic brain measurements (all treated groups) and neurohistopathological examination (0 and 10 000 ppm only). Pups not selected for neurohistopathology were sacrificed and evaluated for gross lesions.

- *Subset 2 (up to 25 rats of each sex per group)*: Pups were evaluated for day of preputial separation or vaginal patency and for passive avoidance response on PND 22. Water maze testing, swimming ability, learning and memory were measured on PNDs 58–62. In the passive avoidance and water maze trials, each rat was tested twice, with test sessions separated by a 1-week interval. After completion of the behavioural evaluations, pups were sacrificed and examined for gross lesions.

- *Subset 3 (up to 24 rats of each sex per group)*: Pups were evaluated for day of preputial separation or vaginal patency and motor activity on PNDs 13, 17, 21 and 60–62. The acoustic startle habituation response was measured on PNDs 22 and 59–62. After completion of the behavioural evaluations, pups were sacrificed and examined for gross lesions.

- *Subset 4 (up to 25 rats of each sex per group)*: Pups were evaluated for detailed clinical observations outside the home cage on LDs 4 and 11 and the day of preputial separation or vaginal patency. Ten rats of each sex per group were killed on PND 69 for recording of brain weight/gross dimensions and microscopic brain measurements (all groups) and neurohistopathology examinations (0 and 10 000 ppm only); pups not selected for these procedures were sacrificed and examined for gross lesions.

- *Subset 5*: Pups selected for subset 5 were not individually identified; they were killed on PND 21 and examined for gross lesions. Gross lesions were subjected to histological evaluation.

All P generation female rats survived to scheduled sacrifice, and there were no treatment-related adverse clinical observations. Mean body weight gain at 10 000 ppm was reduced by 11.2% from GD 6 to GD 20, but mean body weights were generally comparable among the dose groups

(Table 50). Body weight gains during the lactation period did not differ significantly among the exposure groups. Treatment at up to 3000 ppm during gestation and lactation did not affect body weight gains or body weights. Feed consumption was generally comparable among the treated and control groups throughout gestation and lactation. Pregnancy incidences were 92%, 100%, 92% and 96% at 0, 1000, 3000 and 10 000 ppm, respectively, and all pregnant dams delivered litters. All natural delivery observations and litter parameters were comparable in all treated and control groups. Although a statistically significantly lower viability index and significantly higher pup mortality from LD 1 to LD 4 occurred at 3000 ppm, they were considered unrelated to treatment, as the values at 10 000 ppm were comparable to the control values. No clinical or necropsy observations in the F_1 generation pups were attributed to maternal exposure to the test substance at any dose level. No maternal gross lesions related to treatment were evident at necropsy.

Table 50. Selected maternal and litter data in the developmental neurotoxicity study in rats

	0 ppm	1000 ppm	3000 ppm	10 000 ppm
Maternal body weight (g)				
- GD 0	235	235	235	236
- GD 20	391	385	390	378
- LD 0	298	293	297	294
- LD 11	337	324	335	325*
- LD 16	350	340	349	335*
- LD 21	331	323	331	325
Maternal body weight gain (g)				
- GDs 0–6	34.9	34.5	37.6	36.0
- GDs 6–9	14.2	14.8	12.0	7.7**
- GDs 9–12	17.5	17.4	18.3	17.2
- GDs 12–15	20.9	17.7	21.4	19.5
- GDs 15–18	37.6	36.6	36.4	34.1
- GDs 18–20	30.1	30.0	28.3	28.3
- GDs 6–20	120.3	116.4	116.4	106.8**
- LDs 0–21	31.8	28.8	34.2	33.0
No. of rats pregnant/delivering a litter	23/23	25/25	23/23	24/24
Duration of gestation (days)	22.6	22.7	22.5	22.5
Mean no. of implantation sites/litter	14.9	14.5	15.0	14.5
Dams with stillborn pups	1	3	3	2
Litters with 1 or more liveborn pups	23	25	23	24
Mean no. of liveborn pups/litter	14.3	13.7	13.8	13.3
Mean no. of stillborn pups/litter	0.0	0.1	0.1	0.1
Mean litter size, LD 0	14.3	13.7	13.7	13.3
Viability index (%)	99.4	98.5	95.9**	99.1
Lactation index (%)	100	100	100	99.0
Surviving pups/litter, LD 21	10.0	10.0	9.5	9.9
Sex ratio (% males), LD 21	48.1	50.0	49.0	49.5
Mean pup weight/litter (g), LD 0	6.6	6.6	6.5	6.6
Mean pup weight/litter (g), LD 21	50.2	48.6	51.3	50.2

From Hoberman (2010)
* $P < 0.05$; ** $P < 0.01$

In F_1 generation offspring retained beyond weaning for further investigation, there was no treatment-related mortality at any dose level. Two females at 10 000 ppm were found dead, and two control females were missing or killed due to poor clinical condition. All other animals survived to scheduled sacrifice. All clinical observations, including the detailed clinical observations outside the home cage on PNDs 4 and 11, in the F_1 offspring from all treated groups were considered unrelated to treatment, and evaluation of autonomic functions did not reveal any treatment-related effects. Statistically significant differences between the treated and control groups were confined to a higher incidence of umbilical hernia in females at 3000 ppm and a higher incidence of red- or black-coloured tail/tip of tail. There were no treatment-related necropsy observations at any dose level.

In F_1 generation rats, there was no effect of treatment at any dose level on brain weight or cerebral and cerebellar lengths evaluated in subsets 1 and 4 at PNDs 21 and 69, respectively. All group mean values were comparable to, and not significantly different from, control values, with the exception of 1000 ppm males on PND 21, which showed significantly lower mean brain weight compared with controls. Mean brain weights in males at 3000 or 10 000 ppm were comparable to the control value.

In F_1 generation rats, body weights and body weight gains were unaffected by treatment at all dose levels. A single significant increase in body weight gain of subset 4 male rats at 10 000 ppm occurred on PNDs 28–35 but was considered unrelated to treatment, because the increase did not persist and a similar increase was not evident in the other subsets evaluated. Feed consumption was not affected by treatment at any dose level. Statistically significant differences that occurred were considered not to be treatment related because the differences were not dose dependent and/or did not persist.

There was no effect of treatment on the day of vaginal patency or preputial separation in subsets 2, 3 and 4. The mean day of occurrence for each sex was comparable between the treated and control groups, and there were no statistically significant differences. For each sex, group mean body weights at these events were comparable in all treated and control groups.

In subset 2, there were no treatment-related effects on passive avoidance evaluated at PND 22 and again 1 week later or in a water maze test at PNDs 58–62 and again 1 week later. In the passive avoidance test, the mean number of trials to response, the latency of response and the numbers of animals failing to learn were comparable in all treated and control groups, and none of the values for the treated groups was significantly different from the control values. In the water maze performance assessment, the number of trials to achieve criterion, errors per trial, the numbers failing to learn and the latency periods were comparable in all treated and control groups, and none of the values for the treated groups was significantly different from the control values.

In subset 3, motor activity evaluated on PNDs 13, 17, 21 and 58–62 was not affected by treatment. All group mean values for the individual 10-minute blocks and totals (1 hour) for the number of movements and time spent in movement were comparable between the groups, and there were no statistically significant differences. Also, acoustic startle habituation evaluated on PNDs 22 and 58–62 was not affected by treatment.

There were no statistically significant intergroup differences at any dose level for brain weights or for gross cerebral and cerebellar measures in subsets 1 and 4 at PNDs 21 and 69, respectively. In addition, none of the microscopic brain measurements (frontal cortex, parietal cortex, striatum, corpus callosum, hippocampus and cerebellum) were significantly different from the control values at any dose level on PNDs 22 and 69. No treatment-related microscopic lesions were present in any of the tissues examined in the central and peripheral nervous systems in 22-day-old and adult (PND 69) offspring at 10 000 ppm.

The NOAEL for maternal toxicity was 3000 ppm (equal to 237 mg/kg bw per day), based on reduced body weight gain at 10 000 ppm (equal to 784 mg/kg bw per day). The NOAEL for developmental neurotoxicity was 10 000 ppm, the highest dose tested (Hoberman, 2009).

(c) Immunotoxicity

Mice

In an immunotoxicity study conducted in accordance with USEPA test guideline OPPTS 870.7800, groups of 10 male and 10 female Crl:CD1 (ICR) mice were fed diets containing dinotefuran (purity 97.9%) at a concentration of 0, 1120, 2800 or 7000 ppm (groups 1–4) (equal to 0, 153, 405 and 1053 mg/kg bw per day for males and 0, 223, 581 and 1438 mg/kg bw per day for females, respectively) for 4 weeks. A group of eight mice of each sex (group 5), given five daily oral (gavage) doses of cyclophosphamide at 20 mg/kg bw per day on days 22–26, acted as a positive control group. All animals received a sensitizing intravenous dose of sheep red blood cells on day 25 of the study. Animals were examined twice daily for morbidity or mortality, and a detailed clinical examination was performed weekly. Body weights of groups 1–4 were recorded twice during the week before treatment (day −7 and −4), on the 1st day of test (day 1), twice weekly during the treatment period and at necropsy. Group 5 animals were weighed on the 1st day of cyclophosphamide treatment only (day 22). Feed consumption was recorded weekly, and water consumption was recorded for a 3-day period each week throughout the treatment period for groups 1–4. The spleen from each animal in all groups was used as the source of splenocytes for conducting a plaque-forming cell (PFC) assay using a modification of the Jerne PFC assay, which had been fully validated at the conducting laboratory in addition to the concurrent positive controls. All animals were killed after 4 weeks of treatment and subjected to detailed necropsy. Spleen and thymus were weighed and were also adjusted for terminal body weight. Spleen tissues required for immunotoxicology investigations were retained from groups 1–5 for assessment of the acquired or adaptive immune response using a modification of the Jerne PFC assay.

The whole spleen was transferred to individual containers of Hank's balanced salt solution and held on (water) ice until processed for analysis. Splenocyte suspensions were prepared by mechanical dissociation and used for the PFC assay. All samples were coded and randomized for the assay. Duplicate samples per test per animal were enumerated using a dissection microscope with substage illumination. Viability testing was conducted on spleen cell preparations using a trypan blue dye exclusion method. The numbers of viable and non-viable cells were recorded to enable calculation of both the proportion and number of viable spleen cells per millilitre. The number of lytic plaques for each animal was determined, and group mean responses were calculated as PFCs per spleen and PFCs per 10^6 cells. The number of mononuclear cells per spleen was also calculated.

There were no treatment-related clinical signs at any dose level or in the cyclophosphamide-treated group, and no animals died prematurely. There were no treatment-related effects on body weight gain in either sex at any dose level. Although the overall mean body weight gain of males at 7000 ppm (2.3 g) was lower than the mean control gain (3.8 g), the difference was not statistically significant. Some males from all treated and control groups lost weight following the administration of antigen on day 25, but the loss in males at 7000 ppm was greater than in other groups and largely accounted for the observed difference.

The feed consumption and water consumption of all treated groups were unaffected by treatment with dinotefuran at all dose levels. The mean overall feed consumption of the treated groups was within the range 96–109% of control values, and overall mean water consumption was within the range 86–114%.

Absolute and body weight–adjusted spleen and thymus weights were not significantly different from the control values for all dinotefuran-treated groups of both sexes and were therefore considered not to have been affected by treatment.

The macroscopic examination performed after 4 weeks of treatment revealed no lesions attributable to treatment with dinotefuran. The nature and incidence of all the findings were consistent with the commonly seen background of macroscopic changes in CD-1 strain mice.

There was no effect of treatment at any dose level on the humoral T lymphocyte–dependent antibody response to sheep red blood cells, as measured using the modified PFC assay. There were no statistically significant differences in the dinotefuran-treated groups at any dose level in the number of

cells per spleen, PFCs per 10^6 viable cells and PFCs per spleen, when compared with the vehicle control group.

Treatment with five daily oral doses of cyclophosphamide at 20 mg/kg bw per day on days 22–26 resulted in a marked and statistically significant reduction of the PFC response. The number of cells per spleen, PFCs per 10^6 viable cells and PFCs per spleen were all statistically significantly reduced for males and females when compared with the controls, demonstrating the sensitivity of the PFC assay (Table 51).

Table 51. Summary of selected findings in the immunotoxicity study in mice

	Males					Females				
	0 ppm	1120 ppm	2800 ppm	7000 ppm	CP[a]	0 ppm	1120 ppm	2800 ppm	7000 ppm	CP[a]
Body weight (g)										
- day 1	35.7	36.0	36.0	33.8	—	27.3	26.4	27.4	26.7	—
- day 8	37.1	37.5	37.6	34.8	—	28.5	27.9	28.3	28.1	—
- day 22	39.1	40.6	40.1	36.4	—	30.4	29.8	31.2	28.7	—
- day 29	39.5	39.3	40.4	36.1	—	31.0	30.2	31.3	30.1	—
Body weight gain (g), days 1–29	3.8	3.3	4.4	2.3	—	3.7	3.8	3.9	3.5	—
Final body weight (g)	39.8	39.1	40.3	36.2*	—	30.9	30.0	31.1	29.9	—
Spleen weight (mg)	148	128	151	144	—	162	174	178	170	—
Thymus weight (mg)	41.6	36.5	36.4	31.0	—	54.0	49.6	51.1	51.9	—
Cells/spleen ($\times 10^7$)	9.29	6.40	7.74	7.65	4.84*	7.01	8.35	8.20	7.72	2.91**
PFCs/10^6 viable cells	1456	1729	1547	1519	17***	1197	1588	1135	1603	196***
PFCs/spleen	138 221	114 871	125 781	114 221	839***	96 605	140 864	106 564	129 998	7091***

From Bottomley (2011)
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$
[a] Cyclophosphamide, 20 mg/kg bw per day, by oral gavage, on days 22–26.

The NOAEL for immunotoxicity was 7000 ppm (equal to 1053 mg/kg bw per day for males and 1438 mg/kg bw per day for females), the highest dose tested. The NOAEL for systemic toxicity in males was 2800 ppm (equal to 405 mg/kg bw per day), based on decreased body weight at 7000 ppm. In females, the NOAEL for systemic toxicity was 7000 ppm (equal to 1438 mg/kg bw per day), the highest dose tested (Bottomley, 2011).

Rats

In an immunotoxicity study conducted in accordance with USEPA test guideline OPPTS 870.7800, groups of 10 male and 10 female Crl:CD (SD) rats were fed diets containing dinotefuran (purity 97.9%) at a concentration of 0, 2240, 5600 or 14 000 ppm (groups 1–4) (equal to 0, 164, 425 and 992 mg/kg bw per day for males and 0, 179, 430 and 1018 mg/kg bw per day for females) for 4 weeks. A group of eight rats of each sex (group 5), given a single intraperitoneal dose of

cyclophosphamide at 50 mg/kg bw on day 27, acted as a positive control group. All animals received a sensitizing intravenous dose of sheep red blood cells on day 25 of the study. Animals were examined twice daily for morbidity or mortality, and a detailed clinical examination was performed weekly. Body weights of groups 1–4 were recorded twice during the week before treatment (days −7 and −4), on the 1st day of test (day 1), twice weekly during the treatment period and at necropsy. Group 5 animals were weighed on the day of cyclophosphamide treatment only (day 27). Feed consumption was recorded weekly, and water consumption was recorded for a 3-day period each week throughout the treatment period for groups 1–4. The spleen from each animal in all groups was used as the source of splenocytes for conducting a PFC assay using a modification of the Jerne PFC assay, which had been fully validated at the conducting laboratory in addition to the concurrent positive controls. All animals were killed after 4 weeks of treatment and subjected to detailed necropsy. Spleen and thymus were weighed and were also adjusted for terminal body weight. Spleen tissues required for immunotoxicology investigations were retained from groups 1–5 for assessment of the acquired or adaptive immune response using a modification of the Jerne PFC assay.

The whole spleen was transferred to individual containers of Hank's balanced salt solution and held on (water) ice until processed for analysis. Splenocyte suspensions were prepared by mechanical dissociation and used for the PFC assay. All samples were coded and randomized for the assay. Duplicate samples per test per animal were enumerated using a dissection microscope with substage illumination. Viability testing was conducted on spleen cell preparations using a trypan blue dye exclusion method. The numbers of viable and non-viable cells were recorded to enable calculation of both the proportion and number of viable spleen cells per millilitre. The number of lytic plaques for each animal was determined, and group mean responses were calculated as PFCs per spleen and PFCs per 10^6 cells. The number of mononuclear cells per spleen was also calculated.

There were no treatment-related clinical signs at any dose level or in the cyclophosphamide-treated group, and no animals died prematurely. The body weight gain of males at 14 000 ppm was persistently lower than control values throughout the treatment period, resulting in an overall (days 1–29) weight gain that was statistically significantly lower than the control value by approximately 28%. On day 29, the group mean body weight of males at 14 000 ppm was 89.7% of the control value. The body weights for both sexes at 2240 and 5600 ppm and females at 14 000 ppm were considered to have been unaffected by treatment. Although the overall weight gain among females at 14 000 ppm was 10% lower than the control value, it did not attain statistical significance and was due to the lower weight gain that occurred between days 1 and 4 of treatment, with the subsequent weight gain being similar to that of controls. Thus, the group mean body weight of females at 14 000 ppm on day 29 remained marginally higher than the control value.

Feed consumption was persistently lower than that of controls for both sexes at 14 000 ppm, resulting in an overall reduction, compared with controls, of 12% in males and 10% in females. The feed consumption of both sexes at 2240 and 5600 ppm was considered to be unaffected by treatment. The slightly low feed consumption of females at 5600 ppm represented a trend that was present before treatment commenced and was therefore considered not to be due to treatment with dinotefuran. Water consumption among all treated groups of both sexes, although variable, was similar to control or pretreatment values and was therefore considered to be unaffected by treatment.

The macroscopic examination performed after 4 weeks of treatment revealed no lesions attributable to treatment with dinotefuran. The nature and incidence of all the findings were consistent with the commonly seen background of macroscopic changes in Crl: CD (SD) strain rats. Absolute and body weight–adjusted spleen and thymus weights were similar to, and not significantly different from, the control values for all dinotefuran-treated groups of both sexes and were therefore not affected by treatment.

There was no effect of treatment at any dose level on the humoral T lymphocyte–dependent antibody response to sheep red blood cells, as measured using the modified PFC assay. There were no statistically significant changes in the number of cells per spleen, PFCs per 10^6 viable cells or PFCs per spleen, when compared with the controls, for the treated groups that received dinotefuran at 2240, 5600 or 14 000 ppm.

Treatment with a single dose of cyclophosphamide at 50 mg/kg bw on day 27 resulted in a marked and statistically significant reduction of the PFC response. The number of cells per spleen, PFCs per 10^6 viable cells and PFCs per spleen were all statistically significantly reduced for males and females when compared with the controls, demonstrating the sensitivity of the PFC assay (Table 52).

Table 52. Summary of selected findings in the immunotoxicity study in rats

	Males					Females				
	0 ppm	2240 ppm	5600 ppm	14 000 ppm	CP[a]	0 ppm	2240 ppm	5600 ppm	14 000 ppm	CP[a]
Body weight (g)										
- day 1	295	298	303	292	—	210	210	208	219	—
- day 8	343	342	355	322	—	223	224	220	227	—
- day 22	412	408	421	375	—	244	248	237	248	—
- day 29	447	439	460	401	—	253	255	249	258	—
Body weight gain (g), days 1–29	152	141	157	109**	—	44	45	40	39	—
Feed consumption (g/week)										
- week −1	191	191	178	186	—	134	126	123	141	—
- week 1	193	188	203	171	—	133	128	124	116	—
- week 2	196	194	207	174	—	143	137	124	122	—
- week 3	188	183	197	166	—	129	132	120	123	—
- week 4	199	193	208	176	—	136	129	124	123	—
Final body weight (g)	447	439	458	399**	—	252	254	248	257	—
Spleen weight (mg)	872	901	925	772	—	614	599	528	611	—
Thymus weight (mg)	530	527	486	455	—	385	363	343	387	—
Cells/spleen ($\times 10^7$)	48.3	45.9	48.2	45.3	9.7***	37.0	40.4	29.8	34.0	8.0***
PFCs/10^6 viable cells	291	561	609	405	6.9***	814	757	763	626	29.4***
PFCs/spleen	147 252	256 969	290 493	186 292	672***	334 615	296 590	224 822	230 603	2339***

From Chambers (2011)
** $P < 0.01$; *** $P < 0.001$
[a] Cyclophosphamide, 50 mg/kg bw, by intraperitoneal injection, on day 27.

The NOAEL for immunotoxicity was 14 000 ppm (equal to 992 mg/kg bw per day for males and 1018 mg/kg bw per day for females), the highest dose tested. The NOAEL for systemic toxicity was 5600 ppm (equal to 425 mg/kg bw per day in males and 430 mg/kg bw per day in females), based on reduced body weight and body weight gain in males and reduced feed consumption in both sexes at 14 000 ppm (Chambers, 2011).

(d) Transfer into milk of lactating rats

In a study conducted to provide information on the transfer of ^{14}C-labelled dinotefuran and/or its radiolabelled metabolites into the milk of lactating rats, groups of six lactating Sprague-Dawley rats were administered ^{14}C-labelled dinotefuran (purity 97.3%; radiochemical purity 96.0%) as a single dose by oral gavage at a dose level of 50 or 500 mg/kg bw on LDs 2, 4, 8 and 12. The concentration of radioactivity in maternal milk, whole blood and plasma was determined in three rats of each group at each of two time points after each oral dose, 0.5 and 1.5 hours (50 mg/kg bw) and 2 and 4 hours (500 mg/kg bw). Only dams with at least eight viable pups were selected for study, and the number of pups in each litter was reduced to 12 by random selection on LD 1 (the day after completion of parturition). Clinical signs and unusual behaviour of animals were visually checked each working day. Individual body weights were recorded at the start of acclimatization, just before the administration on LDs 2, 4, 8 and 12 and prior to sacrifice. The total weight of the litters was determined on LDs 2, 4, 8 and 16. Three hours prior to milking, the dams were separated from the pups, and milk injection into the mammary glands was stimulated by an intraperitoneal injection of oxytocin (4 international units [IU]/kg bw) about 5 minutes prior to milking. The milk specimen was obtained by a vacuum-driven milking pump at the specified time point from three animals each, and then dams were placed back with their pups. After milking, a 0.5 ml blood sample was withdrawn from the sublingual vein from each animal at the selected time point. Radioactivity was measured by LSC equipped for computing quench-corrected disintegrations per minute.

The range of the average dose administered to the six lactating dams at a nominal dose level of 50 or 500 mg/kg bw was 48.1–51.7 mg/kg bw and 491–509 mg/kg bw, respectively. No unusual appearance or behaviour of pups and dams was observed during the experimental period. Parturition in all pregnant females occurred on day 22 or 23 of pregnancy. The weight gains of the lactating females and their litters were considered to be within the normal range.

In the low-dose group (Table 53), the test substance was rapidly absorbed from the gastrointestinal tract into the systemic circulation. The concentrations in blood, plasma and milk 0.5 hour after administration showed very little variation between sampling occasions from LD 2 to LD 12. The mean values for whole blood and plasma were within the range 30.1–35.2 ppm dinotefuran equivalents, but the concentrations in milk, which were within the range 55.2–62.9 ppm, were approximately 2-fold higher than the whole blood/plasma concentrations. Within 1.5 hours after administration, the concentrations in blood and plasma had declined to about half the levels determined after 0.5 hour and were within the range 13.9–17.5 ppm dinotefuran equivalents. The concentrations in milk were within the range 26.4–36.9 ppm dinotefuran equivalents and remained approximately 2-fold higher than blood and plasma concentrations at all sampling occasions. The achieved concentrations in blood and plasma were very similar at all sampling time points during the observed lactation period, whereas the concentrations in milk showed slightly increasing values.

In the high-dose group (Table 53), the absorption and depletion profiles resembled those observed for the low-dose group. However, the measured concentrations in blood and plasma were only approximately 4 times higher for a 10-fold increase in dose level. Thus, 2 hours after administration, the concentrations in blood and plasma were within the range 104–144 ppm dinotefuran equivalents, and the concentrations in milk were 160–199 ppm dinotefuran equivalents, on all sampling occasions from LD 2 to LD 12. The concentrations in blood and plasma were very similar, and milk concentrations were 40–68% higher than the average concentration in blood and plasma on all sampling occasions. Four hours after administration, the concentrations in blood and plasma remained very similar but had declined to 70–96 ppm dinotefuran equivalents. Milk concentrations had declined to 114–196 ppm and were 50–161% higher than blood/plasma concentrations on all sampling occasions from LD 2 to LD 12. The levels of radioactivity in blood and plasma were not influenced by the period of lactation, but concentrations in milk at 4 hours tended to increase as lactation progressed. However, at this time on LD 12, the measured value showed a high interindividual variation, and the correlation to the blood concentration was significantly different from that at all other time points. Therefore, it was considered likely that the high concentration in milk was partially caused by contamination with urine, as at this time point the posterior-most teats were used for milk sampling.

Table 53. Concentration of total radioactivity in blood, plasma and milk in the transfer-into-milk study in rats

	Concentration of total radioactivity (ppm dinotefuran equivalents)							
	Dosed on LD 2		Dosed on LD 4		Dosed on LD 8		Dosed on LD 12	
Low-dose group								
Dose (mg/kg bw)	*51.7*	*51.3*	*49.8*	*50.7*	*48.1*	*48.5*	*50.5*	*50.8*
Sampling time (h)	*0.5*	*1.5*	*0.5*	*1.5*	*0.5*	*1.5*	*0.5*	*1.5*
Blood	33.1	16.6	30.5	15.3	30.1	13.9	31.2	14.8
Plasma	35.2	17.5	32.5	16.1	32.2	14.8	33.7	15.8
Milk	60.0	26.4	55.2	27.8	58.7	30.7	62.9	36.9
High-dose group								
Dose (mg/kg bw)	*491*	*495*	*509*	*492*	*492*	*492*	*496*	*492*
Sampling time (h)	*2*	*4*	*2*	*4*	*2*	*4*	*2*	*4*
Blood	136	90	104	74	108	70	106	72
Plasma	144	96	109	77	114	75	112	77
Milk	199	141	160	114	187	136	178	196

From Hassler (2006)

In conclusion, the study provides evidence of neonatal exposure of suckling pups to dinotefuran and/or metabolites via the maternal milk (Hassler, 2006).

3. Observations in humans

There were no reports of adverse health effects in manufacturing plant personnel. Also, there were no reports of poisonings with dinotefuran.

Comments

Biochemical aspects

In rats given (^{14}C-tetrahydrofuran)-labelled or (^{14}C-guanidine)-labelled dinotefuran orally by gavage, absorption was rapid and accounted for at least 88% of the total administered radioactivity after a single low dose (50 mg/kg bw) or high dose (1000 mg/kg bw). The maximum plasma concentrations of radioactivity were reached after approximately 0.5 and 2 hours after administration of the low and high doses, respectively, whereas the half-lives in plasma ranged from 4 to 15 hours for the low and high doses, respectively. Radioactivity was widely distributed throughout the body. Elimination of the radioactivity was mainly via urine (≥ 88% of the administered dose), whereas elimination via faeces accounted for 1–3% after oral administration and 1% after intravenous administration. Residues in tissues 168 hours after a single oral or intravenous dose as well as after repeated oral dosing accounted for less than 0.5% of the administered radioactivity, and the concentrations in most tissues were below the limit of detection (0.001 ppm).

Metabolism of dinotefuran in rats was limited, with more than 90% of the dose being eliminated as unchanged parent molecule, which was also the major component in plasma, milk, bile and most tissues collected 4–8 hours after administration. About 20 metabolites were identified; the metabolic routes included hydroxylation on the tetrahydrofuran ring, followed by further oxidation, reduction and acetylation. Other routes of metabolism involved desmethylation, nitro-reduction and hydrolysis.

Toxicological data

The LD_{50} in rats treated orally with dinotefuran was 2450 mg/kg bw. The dermal LD_{50} in rats was greater than 2000 mg/kg bw, and the inhalation LC_{50} in rats was greater than 4.09 mg/l. Dinotefuran was not a skin irritant in rabbits, was slightly irritating to the eye of rabbits and was not a skin sensitizer in the maximization test in guinea-pigs.

Although dinotefuran is neurotoxic in insects, neurotoxicity in mammals was not a critical effect after repeated exposure. No specific target organs were clearly identified in any species following short-term or long-term oral exposure, despite the administration of very high doses of up to 10 635, 3156 and 862 mg/kg bw per day for 13 weeks in mice, rats and dogs, respectively. In all species, the NOAELs were based on decreases in body weight and/or body weight gain as the critical effect. At higher dose levels, a number of minor effects on clinical chemistry parameters, without histopathological correlates, occurred in all species and comprised increased serum albumin concentration and reduced urinary pH in mice, increased serum cholesterol and urea nitrogen concentrations and reduced serum glucose and protein concentrations in rats, and reduced urinary pH in dogs.

In a 4-week study in mice, the NOAEL was 5000 ppm (equal to 901 mg/kg bw per day), based on reduced body weight gain at 25 000 ppm (equal to 4612 mg/kg bw per day) and above. In a 13-week study in mice, the NOAEL was 25 000 ppm (equal to 4442 mg/kg bw per day), based on reduced body weight and body weight gain at 50 000 ppm (equal to 10 635 mg/kg bw per day).

In a 4-week study in rats, the NOAEL was 5000 ppm (equal to 390 mg/kg bw per day), based on reduced body weight gain and increased serum cholesterol in males at 25 000 ppm (equal to 1814 mg/kg bw per day) and above. In a 13-week study in rats, the NOAEL was 500 ppm (equal to 38 mg/kg bw per day), based on reduced body weight and body weight gain at 5000 ppm (equal to 384 mg/kg bw per day) and above in females.

In a 13-week feeding study in dogs, a NOAEL could not be identified in females; the LOAEL was 1600 ppm (equal to 58 mg/kg bw per day) in females, based on a reduction in feed consumption, body weight and body weight gain at all doses administered. In males, the NOAEL was 8000 ppm (equal to 307 mg/kg bw per day), based on a reduction in feed and water consumption, body weight and body weight gain at 24 000–40 000 ppm (equal to an average dose of 862 mg/kg bw per day). In a 1-year feeding study in dogs, the NOAEL was 640 ppm (equal to 22 mg/kg bw per day) in females, based on a reduction in feed consumption, body weight and body weight gain at 3200 ppm (equal to 108 mg/kg bw per day) and above. In males, the NOAEL was 3200 ppm (equal to 111 mg/kg bw per day), based on a reduction in body weight gain at 16 000 ppm (equal to 559 mg/kg bw per day).

Long-term studies of toxicity and carcinogenicity were conducted in mice and rats. In a 78-week study of carcinogenicity in mice, there was no evidence for carcinogenicity up to the highest dose tested (25 000 ppm, equal to 3694 mg/kg bw per day). The NOAEL for toxicity was 2500 ppm (equal to 345 mg/kg bw per day), based on reduced body weight and body weight gain at 25 000 ppm.

In a 104-week study of toxicity and carcinogenicity in rats, there was no evidence for carcinogenicity up to the highest dose tested (20 000 ppm, equal to 991 mg/kg bw per day). The NOAEL for toxicity was 2000 ppm (equal to 100 mg/kg bw per day), based on a reduction in body weight, body weight gain and feed consumption at 20 000 ppm.

The Meeting concluded that dinotefuran is not carcinogenic in mice or rats.

Dinotefuran was tested for genotoxicity in vitro and in vivo in an adequate range of assays. It was not found to be genotoxic.

The Meeting concluded that dinotefuran is unlikely to be genotoxic.

In view of the lack of genotoxicity and the absence of carcinogenicity in rats and mice, the Meeting concluded that dinotefuran is unlikely to pose a carcinogenic risk to humans.

In a two-generation study of reproductive toxicity in rats, the NOAEL for reproductive toxicity was 10 000 ppm (equal to 822 mg/kg bw per day), the highest dose tested, whereas a reduced

number of implantations, increased post-implantation loss and reduced litter size were observed in a range-finding study at 20 000 ppm (equal to 1340 mg/kg bw per day). The NOAEL for parental toxicity was 3000 ppm (equal to 241 mg/kg bw per day), based on a reduction in feed consumption, body weight and spleen weight at 10 000 ppm. The NOAEL for offspring toxicity was 3000 ppm, based on reduced pup weight gain during lactation and reduced spleen weight at 10 000 ppm.

In a developmental toxicity study in rats, the NOAEL for maternal toxicity was 300 mg/kg bw per day, based on decreased weight gain and feed consumption and increased water consumption at 1000 mg/kg bw per day. The NOAEL for developmental toxicity was 1000 mg/kg bw per day, the highest dose tested.

In a developmental toxicity study in rabbits, acute clinical signs (hypoactivity, prone position, panting, erythema, tremor) were observed in dams at 300 mg/kg bw per day from the start of treatment at GD 6 until GD 13; the NOAEL was 125 mg/kg bw per day. Also in dams, a reduction in body weight gain was noted at 125 mg/kg bw per day and above, with a NOAEL of 52 mg/kg bw per day. The NOAEL for developmental toxicity was 300 mg/kg bw per day, the highest dose tested.

The Meeting concluded that dinotefuran was not teratogenic in rats or rabbits.

In an acute neurotoxicity study in rats, the NOAEL was 750 mg/kg bw, based on a transient decrease in motor activity at 1500 mg/kg bw. There was no evidence for neuropathological effects up to the highest dose tested (1500 mg/kg bw).

In a 13-week neurotoxicity study in rats, a transient decrease in motor activity was observed in females at 50 000 ppm (equal to 3806 mg/kg bw per day), which is well above the limit dose. The NOAEL for the study was 5000 ppm (equal to 327 mg/kg bw per day), based on reduced body weight gain and feed consumption at 50 000 ppm (equal to 3413 mg/kg bw per day).

In a dose range–finding developmental neurotoxicity and immunotoxicity study in rats, there was no evidence for developmental neurotoxicity or immunotoxicity up to the highest dose tested (10 000 ppm, equal to 1043 mg/kg bw per day). The NOAEL for maternal toxicity was 10 000 ppm (equal to 670 mg/kg bw per day), the highest dose tested, whereas the NOAEL for offspring toxicity was 3000 ppm (equal to 311 mg/kg bw per day), based on reduced body weight and body weight gain at 10 000 ppm.

In a developmental neurotoxicity study in rats, there was no evidence for developmental neurotoxicity up to the highest dose tested (10 000 ppm, equal to 784 mg/kg bw per day). The NOAEL for maternal toxicity was 3000 ppm (equal to 237 mg/kg bw per day), based on reduced body weight gain at 10 000 ppm.

In 4-week immunotoxicity studies in mice and rats, there was no evidence for immunotoxicity up to the highest dose tested (7000 ppm in mice, equal to 1053 mg/kg bw per day; 14 000 ppm in rats, equal to 992 mg/kg bw per day). The NOAELs for systemic toxicity were 2800 and 5600 ppm (equal to 405 and 425 mg/kg bw per day, respectively) in mice and rats, respectively, based on decreased body weight gain at 7000 and 14 000 ppm, respectively.

There were no reports of adverse health effects in manufacturing plant personnel. Also, there were no reports of poisonings with dinotefuran.

The Meeting concluded that the existing database on dinotefuran was adequate to characterize the potential hazards to fetuses, infants and children.

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) for dinotefuran of 0–0.2 mg/kg bw, based on the NOAEL of 22 mg/kg bw per day for reduced body weight/body weight gain in female dogs in a 1-year toxicity study and application of a safety factor of 100.

The Meeting established an acute reference dose (ARfD) for dinotefuran of 1 mg/kg bw, based on the NOAEL of 125 mg/kg bw for acute clinical signs observed in dams after a single dose of 300 mg/kg bw in a developmental toxicity study in rabbits. A 100-fold safety factor was applied.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen-month study of toxicity and carcinogenicity[a]	Toxicity	2500 ppm, equal to 345 mg/kg bw per day	25 000 ppm, equal to 3694 mg/kg bw per day
		Carcinogenicity	25 000 ppm, equal to 3694 mg/kg bw per day[b]	—
Rat	Two-year study of toxicity and carcinogenicity[a]	Toxicity	2000 ppm, equal to 100 mg/kg bw per day	20 000 ppm, equal to 991 mg/kg bw per day
		Carcinogenicity	20 000 ppm, equal to 991 mg/kg bw per day[b]	—
	Two-generation study of reproductive toxicity[a]	Reproductive toxicity	10 000 ppm, equal to 822 mg/kg bw per day[b]	—
		Parental toxicity	3000 ppm, equal to 241 mg/kg bw per day	10 000 ppm, equal to 822 mg/kg bw per day
		Offspring toxicity	3000 ppm, equal to 241 mg/kg bw per day	10 000 ppm, equal to 822 mg/kg bw per day
	Developmental toxicity study[c]	Maternal toxicity	300 mg/kg bw per day	1000 mg/kg bw per day
		Embryo and fetal toxicity	1000 mg/kg bw per day[b]	—
Rabbit	Developmental toxicity study[c]	Maternal toxicity	125 mg/kg bw per day[d] 52 mg/kg bw per day[e]	300 mg/kg bw per day 125 mg/kg bw per day
		Embryo and fetal toxicity	300 mg/kg bw per day[b]	—
Dog	Thirteen-week and 1-year studies of toxicity[a,f]	Toxicity	640 ppm, equal to 22 mg/kg bw per day	1600 ppm, equal to 58 mg/kg bw per day

[a] Dietary administration.
[b] Highest dose tested.
[c] Gavage administration.
[d] NOAEL for acute clinical signs.
[e] NOAEL for maternal toxicity.
[f] Two studies combined.

Estimate of acceptable daily intake for humans

0–0.2 mg/kg bw

Estimate of acute reference dose

1 mg/kg bw

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposure

Critical end-points for setting guidance values for exposure to dinotefuran

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid; ≥ 88%
Dermal absorption	No data

Distribution	Widely distributed; highest concentrations in kidney and urine
Potential for accumulation	None
Rate and extent of excretion	≥ 93% within 168 h (≥ 88% in urine; 1–3% in faeces; 1–6% in cage rinse)
Metabolism in animals	Limited (> 90% eliminated as parent); hydroxylation on the tetrahydrofuran ring, followed by oxidation, reduction and acetylation; other routes include desmethylation, nitro-reduction and hydrolysis
Toxicologically significant compounds in animals, plants and the environment	Dinotefuran
Acute toxicity	
Rat, LD_{50}, oral	2450 mg/kg bw
Rat, LD_{50}, dermal	> 2000 mg/kg bw
Rat, LC_{50}, inhalation	> 4.09 mg/l (4 h, nose-only exposure)
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Slightly irritating
Dermal sensitization	Not sensitizing (maximization test)
Short-term studies of toxicity	
Target/critical effect	Reduced body weight gain
Lowest relevant oral NOAEL	22 mg/kg bw per day (1-year study in dogs)
Lowest relevant dermal NOAEL	1000 mg/kg bw per day (28-day study in rats)
Lowest relevant inhalation NOAEC	0.22 mg/l (28-day study in rats)
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Reduced body weight gain
Lowest relevant NOAEL	100 mg/kg bw per day (2-year study in rats)
Carcinogenicity	Not carcinogenic in mice or rats
Genotoxicity	
	Not genotoxic
Reproductive toxicity	
Reproduction target/critical effect	No reproductive toxicity; reduced pup weight gain and reduced spleen weight at parentally toxic dose
Lowest relevant reproductive NOAEL	822 mg/kg bw per day (highest dose tested)
Lowest relevant parental NOAEL	241 mg/kg bw per day
Lowest relevant offspring NOAEL	241 mg/kg bw per day
Developmental toxicity	
Developmental target/critical effect	No evidence for developmental toxicity (rats and rabbits)
Lowest relevant maternal NOAEL	52 mg/kg bw per day (rabbits)
Lowest relevant embryo/fetal NOAEL	300 mg/kg bw per day (rabbits) (highest dose tested)
Neurotoxicity	
Acute and subchronic neurotoxicity	No specific signs of acute or subchronic neurotoxicity at highest dose tested (acute: 1500 mg/kg bw; subchronic: 3806 mg/kg bw per day)
Developmental neurotoxicity	No evidence for developmental neurotoxicity at highest dose tested (784 mg/kg bw per day)

Other toxicological studies	
Immunotoxicity	No evidence for immunotoxicity at highest dose tested (1053 mg/kg bw per day in mice; 992 mg/kg bw per day in rats)
Developmental immunotoxicity	No evidence for developmental immunotoxicity at highest dose tested (1043 mg/kg bw per day)
Medical data	
	No adverse health effects reported in manufacturing plant personnel

Summary

	Value	Study	Safety factor
ADI	0–0.2 mg/kg bw	One-year study of toxicity in dogs	100
ARfD	1 mg/kg bw	Developmental toxicity study in rabbits (acute clinical signs in dams)	100

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Appendix 1. Dinotefuran and metabolite key

Code name	IUPAC name CAS No. Formula Molecular weight	Structure	Where found
Dinotefuran MTI-446	(*RS*)-1-Methyl-2-nitro-3-(tetrahydro-3-furylmethyl)guanidine CAS No.: 165252-70-0 Formula: $C_7H_{14}N_4O_3$ MW: 202.11		Parent molecule
UF	1-Methyl-3-(tetrahydro-3-furylmethyl)urea CAS No.: not given Formula: $C_7H_{14}N_2O_2$ MW: 158.20		Metabolism: apple, potato, rice, rape, lettuce, rat
DN phosphate	1-Methyl-3-(tetrahydro-3-furylmethyl)guanidinium dihydrogen phosphate CAS No.: not given Formula: $C_7H_{18}N_3O_5P$ MW: 255.10		Metabolism: apple, rice, rape, lettuce, rat
446-DO	1-[4-Hydroxy-2-(hydroxymethyl)butyl]-3-methyl-2-nitroguanidine CAS No.: not given Formula: $C_7H_{16}N_4O_4$ MW: 220.12		Metabolism: apple, potato, rice, lettuce, rat
446-CO	1-Methyl-2-nitro-3-(2-oxotetrahydro-3-furylmethyl)guanidine CAS No.: not given Formula: $C_7H_{12}N_4O_4$ MW: 216		Metabolism: rat
446-OH+COOH	2-(2-Hydroxyethyl)-3-(3-methyl-2-nitroguanidino)propionic acid CAS No.: not given Formula: $C_7H_{14}N_4O_5$ MW: 234		Metabolism: rat

Code name	IUPAC name CAS No. Formula Molecular weight	Structure	Where found
446-DO-Ac	1-[4-Hydroxy-2-(hydroxymethyl)butyl]-3-methyl-2-nitroguanidine acetyl CAS No.: not given Formula: $C_9H_{18}N_4O_5$ MW: 262		Metabolism: rat
446-NU	1-Nitro-2-(tetrahydro-3-furylmethyl)urea CAS No.: not given Formula: $C_6H_{11}N_3O_4$ MW: 189		Metabolism: rat
446-NH_2	2-Amino-1-methyl-3-(tetrahydro-3-furylmethyl)guanidine CAS No.: not given Formula: $C_7H_{16}N_4O$ MW: 172		Metabolism: rat
PHP	6-Hydroxy-5-(2-hydroxyethyl)-1-methyl-1,3-diazinane-2-ylidene-*N*-nitroamine CAS No.: not given Formula: $C_7H_{14}N_4O_4$ MW: 218.21		Metabolism: apple, potato, rice, rape, lettuce, rat
PHP-Ac	6-Hydroxy-5-(2-hydroxyethyl)-1-methyl-1,3-diazinane-2-ylidene-*N*-nitroamine acetyl conjugate CAS No.: not given Formula: $C_9H_{16}N_4O5$ MW: 260		Metabolism: rat
FNG	2-Nitro-1-(tetrahydro-3-furylmethyl)guanidine CAS No.: 168688-99-1 Formula: $C_6H_{12}N_4O_3$ MW: 188.18		Metabolism: apple, potato, rape, rat

Code name	IUPAC name CAS No. Formula Molecular weight	Structure	Where found
FNG-DN	1-(Tetrahydro-3-furylmethyl)guanidine CAS No.: not given Formula: $C_6H_{13}N_3O$ MW: 143		Metabolism: rat
MNG	1-Methyl-2-nitroguanidine CAS No.: not given Formula: $C_2H_6N_4O_2$ MW: 118.09		Metabolism: apple, potato, rice, rape, lettuce, rat
UF-DO	1-[Hydroxy-2-(hydroxymethyl)butyl]-3-methylurea CAS No.: not given Formula: $C_7H_{16}N_2O_3$ MW: 176.21		Metabolism: apple
UF-DM	1-(Tetrahydro-3-furylmethyl)urea CAS No.: not given Formula: $C_6H_{12}N_2O_2$ MW: 144.17		Metabolism: rat
DN-OH	1-[(5-Hydroxyoxolan-3-yl)methyl]-3-methylurea CAS No.: not given Formula: $C_7H_{15}N_3O_2$ MW: 173.12		Metabolism: rice, lettuce, rat
DN-DO	1-[4-Hydroxymethyl)butyl]-3-methylguanidine CAS No.: not given Formula: $C_7H_{18}N_3O_2$ MW: 175		Metabolism: rat
DN-CO	1-Methyl-3-(2-oxotetrahydro-3-furylmethyl)guanidine CAS No.: not given Formula: $C_7H_{13}N_3O_2$ MW: 171		Metabolism: rat

Code name	IUPAC name CAS No. Formula Molecular weight	Structure	Where found
BCDN succinate	3-(Methylamino)-9-oxa-2-aza-4-azoniabicyclo[4.3.0]non-3-ene hydrogen succinate CAS No.: none given Formula: $C_{11}H_{19}N_3O_5$ MW: 273.13		Metabolism: apple, rice, rape, lettuce, rat
MG hydrogen chloride	1-Methylguanidinium chloride CAS No.: 22661-87-6 Formula: $C_2H_8ClN_3$ MW: 109.04		Metabolism: apple, rice, rape, rat
MG-Ac	1-Methyl-2-acetylguanidine CAS No.: not given Formula: $C_4H_9N_3O$ MW: 115		Metabolism: rat
NG	Nitroguanidine CAS No.: 556-88-7 Formula: $CH_4N_4O_2$ MW: 104.07		Metabolism: apple, potato, lettuce
MPZ	1-Methyl-5-(1-methylethyl)-1,3,5-triazinane-2-ylidene-*N*-nitroamine CAS No.: none given Formula: $C_7H_{15}N_5O_2$ MW: 201.23		Reference substance
PZ	5-(1-Methylethyl)-1,3,5-triazinane-2-ylidene-*N*-nitroamine CAS No.: none given Formula: $C_6H_{13}N_5O_2$ MW: 187.20		Reference substance

CAS, Chemical Abstracts Service; IUPAC, International Union of Pure and Applied Chemistry; MW, molecular weight

FENPROPATHRIN

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Explanation

Fenpropathrin is the International Organization for Standardization (ISO)–approved name for (*RS*)-α-cyano-3-phenoxybenzyl-2,2,3,3-tetramethylcyclopropanecarboxylate (International Union of Pure and Applied Chemistry), with the Chemical Abstracts Service number 39515-41-8. Fenpropathrin is a synthetic pyrethroid with insecticidal/acaricidal properties. Type I pyrethroids act to induce repetitive firing in a cercal sensory nerve. The poisoning symptoms of Type I compounds include restlessness, incoordination, hyperactivity, prostration and paralysis. Type II pyrethroids are generally acyanophenoxybenzyl pyrethroids. They do not induce repetitive firing and are associated with a different set of symptoms, including a pronounced convulsive phase. Fenpropathrin is a unique compound, in that it appears to have both Type I and Type II properties. It produces repetitive firing but is associated with Type II symptoms.

Fenpropathrin was evaluated previously by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 1993, when an acceptable daily intake (ADI) of 0–0.03 mg/kg body weight (bw)

was established based on a no-observed-adverse-effect level (NOAEL) of 3 mg/kg bw per day from a multigeneration reproductive study in rats, a developmental toxicity study in rats and a 1-year toxicity study in dogs and using a 100-fold safety factor. The establishment of an acute reference dose (ARfD) was not considered by the Meeting in 1993.

Fenpropathrin was reviewed at the present Meeting as part of the periodic review programme of the Codex Committee on Pesticide Residues. Since the last review by JMPR, the following new studies of fenpropathrin have been submitted: acute and subchronic neurotoxicity studies, a developmental neurotoxicity study and an immunotoxicity study. Published studies primarily evaluating the neurotoxicity of fenpropathrin have also been taken into consideration.

Most of the studies do not comply with good laboratory practice (GLP), as most of the data were generated before the implementation of GLP regulations. Overall, the Meeting considered that the database was adequate for the risk assessment.

Evaluation for acceptable daily intake

1. Biochemical aspects

Figure 1 shows the radiolabelling position of fenpropathrin used in the absorption, distribution, metabolism and excretion studies in rats.

Figure 1. Structures of the radiolabelled molecules

COO-CH
CN
O

A. [alcohol-^{14}C]Fenpropathrin, radiolabelled as [phenoxyphenyl-^{14}C]fenpropathrin, also referred to as [^{14}C-benzyl]fenpropathrin

COO-CH
CN
O

B. [acid-^{14}C]Fenpropathrin, radiolabelled as [cyclopropyl-1-^{14}C]fenpropathrin

1.1 Absorption, distribution and excretion

In an absorption, distribution, metabolism and excretion study, six male and six female Charles River CD rats received a single dose of [^{14}C-benzyl]fenpropathrin (radiochemical purity 99.5%) via gavage in corn oil at a dose of 1.5 mg/kg bw. Treated rats were individually housed in glass metabolism cages to facilitate the collection of urine and faeces. Two rats of each sex were sacrificed at 24 hours, 72 hours and 8 days post-treatment. Blood, kidney, liver, brain, guts, skin, fat, muscle and remaining carcass were analysed for radioactivity. In a separate experiment, two female rats were treated similarly and housed individually in glass metabolism cages equipped with a carbon dioxide trap.

Absorption was rapid and excretion almost complete (97%) within 48 hours. About 56% of the administered dose was found in urine and 40% in faeces at 48 hours (Table 1). The amount of radioactivity excreted via expired air was 0.005%. The low residues found in blood, liver, kidney, fat,

muscle and brain 24 hours after dosing depleted rapidly over the following 7 days to barely detectable levels, and less than 1.5% of the administered dose remained in the body 8 days after treatment (Table 2). The highest residue was found in the fat, probably representing a limited retention of a small amount of the lipophilic substance (Crawford, 1975).

Table 1. Excretion of radioactivity following single oral (gavage) dose of 1.5 mg/kg bw in rats

	Radioactivity (% of administered dose)								
	24 h	48 h	72 h	96 h	120 h	144 h	168 h	192 h	Total
Urine									
Males	48.8	6.7	1.3	0.5	0.3	0.2	0.2	0.1	58.1
Females	48.7	7.3	1.4	1.2	0.5	0.4	0.4	0.07	59.97
Faeces									
Males	18.8	20.6	6.6	1.6	0.2	0.1	0.2	0.03	48.13
Females	12.6	17.8	3.2	0.7	0.3	0.2	0.07	2.46	37.33

From Crawford (1975)

Table 2. Distribution of radioactivity in selected tissues following single oral (gavage) dose of 1.5 mg/kg bw in rats

Tissue	Concentration of radioactivity in tissues (µg/g)					
	Day 1		Day 3		Day 8	
	Males	Females	Males	Females	Males	Females
Liver	0.4	0.6	0.03	0.04	0.01	0.01
Kidney	0.1	0.2	0.03	0.02	0.008	0.007
Fat	0.6	0.5	0.3	0.3	0.2	0.2
Muscle	0.01	0.03	0.005	0.002	0.004	0.002
Brain	0.005	0.009	0.003	0.0008	0.003	0.0002
Blood	0.09	0.2	0.008	0.009	0.001	0.001

From Crawford (1975)

Rapid excretion was also noted following a single oral administration of [^{14}C-cyclopropyl]fenpropathrin, although amounts in urine and faeces were in this case approximately the same (40%) (Crawford, 1975; Crawford & Hutson, 1976, 1977).

In a published study, absorption, distribution and excretion following a single low dose or high dose of fenpropathrin were studied in rats. In this study, fenpropathrin labelled with ^{14}C at the benzyl carbon of the alcohol moiety (^{14}C-alcohol) or at the C1 position of the cyclopropane ring of the acid moiety (^{14}C-acid) and 2,2,3,3-tetramethylcyclopropanecarboxylic acid (TMPA) labelled at the C1 position of the cyclopropane ring were used. Five male and five female Sprague-Dawley rats were given radiolabelled fenpropathrin by oral gavage as a single low dose of 2.4–3.8 mg/kg bw or a single high dose of 18.5–26.8 mg/kg bw. Groups of three male Sprague-Dawley rats were given radiolabelled TMPA as a single oral gavage dose of 1.0–1.1 mg/kg bw (calculated as 2.5 mg/kg bw fenpropathrin equivalent). Corn oil was used as the vehicle (5 ml/kg bw) for administration. Urine and faeces were collected at various time points. Treated rats were sacrificed 7 days after the treatment. Selected tissues were analysed for radioactivity.

Single oral administration of [^{14}C-acid]fenpropathrin and [^{14}C-alcohol]fenpropathrin to male and female rats at 2.4–26.8 mg/kg bw resulted in rapid and almost complete elimination of radiocarbon from the body. Radiolabelled recoveries for the acid and alcohol labellings in the two dose groups were 96.4–101.9% (urine: 26.8–43.8%; and faeces: 58.1–70.4%) and 96.4–98.0% (urine: 26.0–42.7%; and faeces: 54.1–71.0%), respectively, in 7 days. Radioactivity excretion patterns of both labelled preparations into the urine and faeces were very similar in the two dose groups. Faecal excretion of radioactivity was higher in the high-dose groups than in the low-dose groups. There was no significant sex difference in the excretion patterns. When rats were orally dosed with [^{14}C]TMPA, radiocarbon was rapidly excreted mainly into the urine (88% of the dose). No sex-related differences in tissue distribution were observed. Radioactivity in tissue residues was generally very low with both labellings, although the levels were slightly higher in the fat (0.1–1.4 parts per million [ppm]). Tissue residues were generally lower with the alcohol labelling than with the acid labelling (Kaneko et al., 1987).

In a 1994 metabolism study, groups of five male and five female Crl:CD®BR® VAF/Plus (Sprague-Dawley) rats were dosed by oral gavage with radiolabelled fenpropathrin by three protocols. The fenpropathrin was radiolabelled on either the alcohol ([alcohol-^{14}C]fenpropathrin; purity 98.7%; lot no. C-91-089) or acid ([acid-^{14}C]fenpropathrin; purity 98.5%; lot no. C-90-039) portion of the molecule. In Experiment I, rats received 14 daily oral low doses of 2.5 mg/kg bw of unlabelled fenpropathrin followed by a 15th dose of either the alcohol- or acid-radiolabelled fenpropathrin. In Experiments II and III, groups of rats received a single dose of either of the two radiolabelled test articles at 2.5 mg/kg bw (II) or 25 mg/kg bw (III). Corn oil (5 ml/kg bw) was used as the vehicle. Urine and faeces were collected up to 168 hours after gavage dosing. Animals were sacrificed at the end of the exposure period, and various tissues were analysed for radioactivity. Urine, faeces and selected tissues were subjected to various analytical methods to identify and quantify metabolites (see section 1.2).

No clinical signs were seen in any rats. The overall recoveries were 93–108% of the administered dose. The excretion of radioactivity in the urine and faeces was very rapid regardless of the sex, the dose or the radiolabel position (Table 3). The half-life for urinary excretion was 11–16 hours, and the half-life for faecal excretion was 7–9 hours. Slightly higher urinary excretion (52–56%) was observed with repeated dosing compared with the single low dose (30–40%) or single high dose (28–35%). Excretion was slightly higher in the faeces than in the urine in this study (Savides et al., 1994), whereas the excretion of radioactivity in the urine was the same as that in the faeces (around 50%) in the Crawford (1975) study at the low dose, as described above. Less than 1% of the administered radiolabelled dose was present in the tissues at termination (168 hours post-dosing), with the highest concentration of radiolabel in the fat (Savides et al., 1994).

1.2 Biotransformation

The rates of absorption, distribution and excretion of (^{14}C-benzyl)- and (^{14}C-cyclopropyl)-labelled fenpropathrin following a single oral gavage dose of 1.5 mg/kg bw were studied in rats (Crawford, 1975; Crawford & Hutson, 1976, 1977). Elimination of (^{14}C-benzyl)- and (^{14}C-cyclopropyl)-labelled fenpropathrin was studied in bile duct–cannulated rats. The identification of metabolites from urine, faeces and bile samples was conducted using various analytical methods. The abbreviations used for metabolites in this section are explained in footnote a to Table 4.

In the Kaneko et al. (1987) study (see section 1.1), about 29–53% of the parent compound was detected in the faeces (Table 4). No parent compound was detected in the urine.

Table 3. Excretion and tissue distribution of fenpropathrin following a single low, single high or repeated dose in rats at 168 hours[a]

Dose	Label	Sex	% of administered dose								
			Urine				Faeces			Tissues	Total
			12 h	24 h	48 h	Total (168 h)	24 h	48 h	Total (168 h)		
Low 2.5 mg/kg bw	Alcohol	M	16.17	15.55	2.98	35.97	58.72	9.07	69.03	0.47	105.47
		F	17.94	16.14	4.43	39.74	56.22	7.89	64.95	0.25	104.94
	Acid	M	12.79	9.99	5.31	29.87	58.44	8.47	68.39	0.67	98.93
		F	17.57	10.56	4.36	34.24	54.28	8.56	64.68	0.52	99.43
High 25 mg/kg bw	Alcohol	M	14.25	13.80	2.63	31.86	58.69	4.97	65.25	0.31	97.41
		F	10.31	12.59	3.82	27.85	53.38	10.72	65.14	0.27	93.26
	Acid	M	11.61	13.67	4.96	32.04	59.82	7.15	68.60	0.52	101.16
		F	12.74	13.98	6.92	35.37	52.53	10.69	64.99	0.42	100.78
Repeated dose 2.5 mg/kg bw	Alcohol	M	31.42	15.27	3.55	52.30	42.27	10.63	54.66	0.97	107.93
		F	36.51	13.35	3.52	55.39	43.49	6.46	51.14	0.71	107.24
	Acid	M	26.13	18.43	5.13	51.85	38.98	10.72	51.89	1.26	105.01
		F	36.50	14.41	3.91	56.47	38.05	7.25	46.47	0.77	103.70

From Savides et al. (1994), pp. 79–81, 89–91 and 99–101

F, female; M, male

[a] Values represent averages for all animals. Repeated dose = 14 doses of unlabelled fenpropathrin (2.5 mg/kg bw) followed by a single radioactive dose.

Table 4. Proportion of metabolites in the 0- to 2-day faeces and urine after single oral administration of (^{14}C-acid)- or (^{14}C-alcohol)-labelled fenpropathrin in male Sprague-Dawley rats at low and high doses

Metabolites[a]	% of administered dose			
	Acid labelling		Alcohol labelling	
	Low dose	High dose	Low dose	High dose
Faeces				
Fenpropathrin	35.5 ± 13.0[b]	48.2 ± 8.8	29.2 ± 11.6	53.0 ± 4.2
PBald	—	—	0.3 ± 0.1	0.3 ± 0.1
PBacid	—	—	0.7 ± 0.5	0.1 ± 0.1
COOH *trans*-Fenp.	1.5 ± 0.3	1.3 ± 0.4	1.9 ± 0.4	1.0 ± 0.1
4′-OH-Fenp.	2.3 ± 1.8	1.8 ± 0.5	2.0 ± 1.0	1.0 ± 0.4
CH_2OH *trans*-Fenp.	4.5 ± 2.0	6.2 ± 1.9	7.9 ± 1.8	5.3 ± 0.6
4′-OH,CH_2OH *trans*-Fenp.	1.9 ± 0.5	1.4 ± 0.4	2.3 ± 0.6	1.3 ± 0.2
Others	8.9 ± 3.0	7.2 ± 2.3	12.2 ± 6.0	4.9 ± 0.8
Unextractable	3.0 ± 0.5	3.1 ± 1.0	4.4 ± 1.0	2.8 ± 0.4
Total	*57.6 ±11.5*	*69.2 ±4.4*	*60.8 ±8.1*	*69.7 ±2.7*
Urine				
PBacid	—	—	2.0 ± 0.6	2.3 ± 0.2
PBacid–glycine	—	—	0.9 ± 0.2	0.8 ± 0.2
4′-OH-PBacid–sulfate	—	—	24.8 ± 5.0	18.7 ± 2.3

Table 4 (continued)

Metabolites[a]	% of administered dose			
	Acid labelling		Alcohol labelling	
	Low dose	High dose	Low dose	High dose
2′-OH-PBacid–sulfate	—	—	0.9 ± 0.3	0.5 ± 0.1
TMPA free	0.4 ± 0.2	0.4 ± 0.4	—	—
TMPA–glucuronide	10.3 ± 3.1	8.6 ± 2.2	—	—
TMPA-COOH (*trans*)	2.1 ± 0.6	1.4 ± 0.3	—	—
TMPA-CH_2OH (*trans*) free	5.4 ± 1.8	3.8 ± 0.5	—	—
TMPA-CH_2OH (*trans*)–glucuronide	0.7 ± 0.2	0.6 ± 0.1	—	—
TMPA-CH_2OH-lactone free	1.2 ± 0.4	1.1 ± 0.3	—	—
TMPA-CH_2OH-lactone–glucuronide	1.6 ± 0.5	1.4 ± 0.4	—	—
Others	11.7 ± 3.6	8.5 ± 1.5	4.0 ± 1.1	2.9 ± 0.5
Total	*33.4 ±9.8*	*25.8 ±4.1*	*32.6 ±6.8*	*25.2 ±3.1*

From Kaneko et al. (1987)

[a] Abbreviations for metabolites:

1. Fenpropathrin = α-cyano-3-phenoxybenzyl 2,2,3,3-tetramethylcyclopropanecarboxylate
2. COOH *trans*-Fenp. = α-cyano-3-phenoxybenzyl 2-carboxy-2,2,3-trimethylcyclopropanecarboxylate
3. 2′-OH-Fenp. = α-cyano-3-(2′-hydroxyphenoxy)benzyl 2,2,3,3-tetramethylcyclopropanecarboxylate
4. 4′-OH-Fenp. = α-cyano-3-(4′-hydroxyphenoxy)benzyl 2,2,3,3-tetramethylcyclopropanecarboxylate
5. CH_2OH *trans*-Fenp. = α-cyano-3-phenoxybenzyl 2-hydroxymethyl-2,3,3-trimethylcyclopropanecarboxylate
6. 2′-OH,CH_2OH *trans*-Fenp. = α-cyano-3-(2′-hydroxyphenoxy)benzyl 2-hydroxymethyl-2,3,3-trimethylcyclopropanecarboxylate
7. 4′-OH,CH_2OH *trans*-Fenp. = α-cyano-3-(4′-hydroxyphenoxy)benzyl 2-hydroxymethyl-2,3,3-trimethylcyclopropanecarboxylate
8. TMPA = 2,2,3,3-tetramethylcyclopropanecarboxylic acid, free or its glucuronide conjugate
9. TMPA-CH_2OH (*trans*) = 2-hydroxymethyl-2,3,3-trimethylcyclopropanecarboxylic acid, free or its glucuronide conjugate
10. TMPA-COOH (*trans*, *cis*) = 2-carboxy-2,3,3-trimethylcyclopropanecarboxylic acid
11. TMPA-lactone = 5,6,6-trimethyl-3-oxabicyclo[3.1.0]hexan-2-one
12. TMPA-$(CH_2OH)_2$ = 2,2-dihydroxymethyl-3,3-dimethylcyclopropanecarboxylic acid
13. TMPA-CH_2OH-lactone = 5-hydroxymethyl-6,6-dimethyl-3-oxabicyclo[3.1.0]hexan-2-one, free or its glucuronide conjugate
14. PBald = 3-phenoxybenzaldehyde
15. 2′-OH-PBacid–sulfate = 3-(2′-hydroxyphenoxy)benzoic acid, sulfate conjugate
16. 4′-OH-PBacid–sulfate = 3-(4′-hydroxyphenoxy)benzoic acid, sulfate conjugate
17. PBacid = 3-phenoxybenzoic acid
18. PBacid–glycine = *N*-3-phenoxybenzoyl-glycine

[b] Mean value of five male rats ± standard deviation.

In the study by Savides et al. (1994) (see section 1.1), urine and faecal samples from 0- to 48-hour collections were pooled together and subjected to metabolic identification. The chromatographic profiles from the faecal extracts were similar, regardless of type of dose, sex or radiolabel position. About 13–34% of the administered dose was excreted as the parent compound (fenpropathrin), about 9–20% as CH_2OH fenpropathrin, about 4–11% as 4′-OH-fenpropathrin and about 2–7% as 4′-OH,CH_2OH fenpropathrin. The chromatographic profiles of the urine from the [acid-^{14}C]fenpropathrin and [alcohol-^{14}C]fenpropathrin groups were different, suggesting that the metabolism of the fenpropathrin molecule involved an ester cleavage between the cyclopropyl and aromatic portions of the molecule. Approximately four metabolites were present in the urine from the [alcohol-^{14}C]fenpropathrin group. All of the major metabolites were identified. The major metabolite in the urine from this group was the sulfate conjugate of 4′-OH-PBacid, representing about 23–44% of

the administered dose. PBacid (1–9% of the administered dose), 4′-OH-PBacid (1–9% of the administered dose) and PBacid–glycine (1–2% of the administered dose) were also excreted in the urine from the [alcohol-^{14}C]fenpropathrin.

The glucuronic acid conjugate of TMPA was positively identified, and TMPA-COOH (*trans*), TMPA-CH_2OH-lactone and TMPA-CH_2OH were tentatively identified in the urine from the [acid-^{14}C]fenpropathrin. Savides et al. (1994) also concluded that the excretion rate, ^{14}C residues and identified metabolites were similar to those obtained in a previous study with fenpropathrin (Crawford & Hutson, 1977). In both the current study and the cited study, predominant biotransformations of fenpropathrin included oxidation at the acid and alcohol moieties, cleavage of the ester linkage and conjugation with sulfuric acid or glucuronic acid (Savides et al., 1994).

On the basis of the identified metabolites, the major biotransformation reactions of fenpropathrin in rats consisted of oxidation at the methyl groups of the acid moiety and at the 2′- and 4′-positions of the alcohol moiety, cleavage of the ester linkage and conjugation of the resultant carboxylic acids, alcohols and phenols with glucuronic acid, sulfuric acid and glycine. Most of the urinary metabolites were ester-cleaved ones. The predominant urinary metabolites derived from the acid moiety were identified as TMPA–glucuronide and TMPA-CH_2OH (*trans*). Other metabolites identified were TMPA-COOH (*trans*) and TMPA-CH_2OH-lactone in free form or as the glucuronide. The major urinary metabolites derived from the alcohol moiety were PBacid in free form and as the glycine conjugate, 4′-OH-PBacid–sulfate and 2′-OH-PBacid–sulfate. The urinary metabolites from the alcohol moiety were similar to those from other pyrethroids (e.g. fenvalerate, deltamethrin, cypermethrin). Almost all faecal metabolites retained the ester linkage. The major faecal metabolite was identified as CH_2OH *trans*-fenpropathrin, followed by COOH *trans*-fenpropathrin, 4′-OH-fenpropathrin and 4′-OH,CH_2OH *trans*-fenpropathrin. Depending on the dose administered, 30–50% of the applied radioactivity was excreted in faeces as parent compound. Fenpropathrin and TMPA were the major components of ^{14}C in tissues. No sex difference was apparent (Kaneko et al., 1987). An aryl-hydroxylated ester (α-cyano-3-(4′-hydroxyphenoxy)benzyl ester) was identified in bile. The ester was eliminated in the bile presumably conjugated (Crawford & Hutson, 1976, 1977).

The proposed metabolic pathway of fenpropathrin in rats is shown in Figure 2.

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

The results of acute toxicity studies with fenpropathrin (including skin and eye irritation and dermal sensitization studies; see below) are summarized in Table 5. All the studies were in compliance with European Union (EU) guidelines of the day and either in compliance with GLP or, where pre-1993, conducted and reported to an adequate standard.

Independent of the route of exposure, onset of toxic signs and lethality are rapid (within a few hours or days, respectively). Recovery in surviving animals is also rapid. Clinical signs of toxicity via oral exposure are indicative of neurotoxicity and include tremors, hindlimb ataxia, decreased spontaneous motor activity, clonic convulsions, salivation, lacrimation, fibrillation and hypersensitivity. In the dog, emesis is also observed; however, the estimated LD_{50} in this species (i.e. > 1000 mg/kg bw; one dog out of the four treated at 1000 mg/kg bw died) appears unreliable, as the subchronic NOAEL and maximum tolerated dose (MTD) are not different from those for other mammalian species.

Non-lethal oral doses range between 10 and 50 mg/kg bw in rats and mice, with the higher value obtained in the same acute oral study for which higher LD_{50} values were determined, and in which 10% gum arabic instead of corn oil was used as the vehicle.

Figure 2. Proposed metabolic pathways of fenpropathrin in mammals

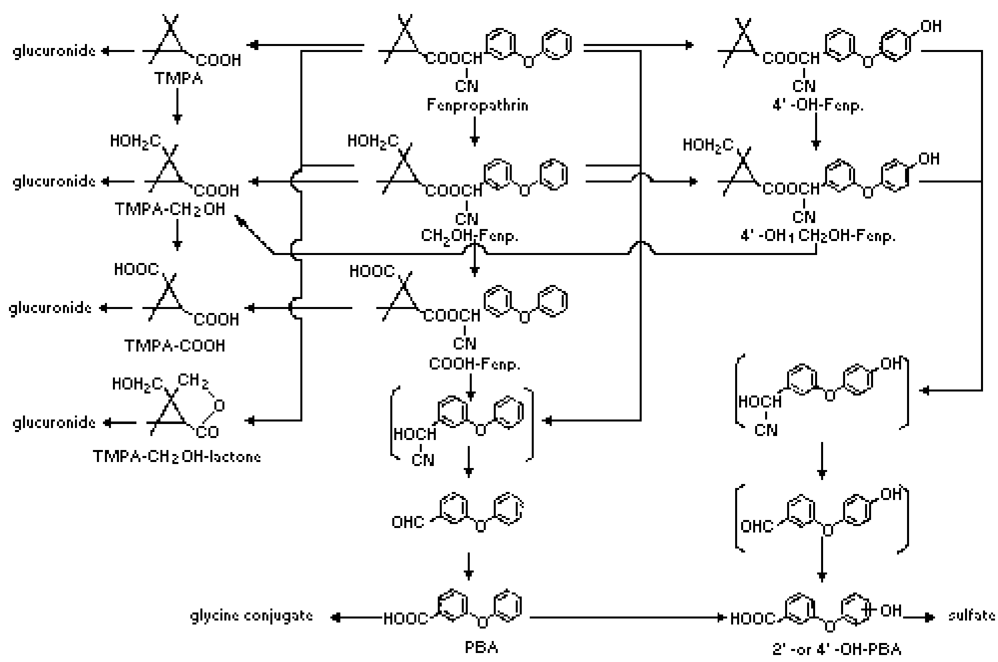

Table 5. Acute toxicity of fenpropathrin

Species	Strain	Sex	Route	Purity; vehicle	Result	Reference
Mouse	dd	M + F	Oral	97.0%; corn oil	LD_{50} M: 67 mg/kg bw F: 58 mg/kg bw	Kohda & Kadota (1975)
Mouse	ICR	M + F	Oral	93.8%; 10% gum arabic	LD_{50} M: 135 mg/kg bw F: 154 mg/kg bw	Suzuki et al. (1986)
Rat	Sprague-Dawley	M + F	Oral	97.0%; corn oil	LD_{50} M: 54.0 mg/kg bw F: 48.5 mg/kg bw	Kohda (1979a)
Rat	Sprague-Dawley	M + F	Oral	97.3%; 10% gum arabic	LD_{50} M: 164 mg/kg bw F: 104 mg/kg bw	Hiromori et al. (1982)
Rat	Sprague-Dawley	M + F	Oral	97.3%; corn oil	LD_{50} M: 77.4 mg/kg bw F: 66.7 mg/kg bw	Hiromori et al. (1983a)
Rat	Sprague-Dawley	M + F	Oral	91.8%; corn oil	LD_{50} M: 70.6 mg/kg bw F: 66.7 mg/kg bw	Hiromori et al. (1983b)
Rat	Sprague-Dawley	M + F	Oral	93.8%; corn oil	LD_{50} M: 60 mg/kg bw F: 70 mg/kg bw	Omodaka et al. (1986a)
Rat	Sprague-Dawley	F	Oral	92.7%; corn oil	LD_{50} > 50 mg/kg bw but < 259 mg/kg bw	Asano (2011a)

Species	Strain	Sex	Route	Purity; vehicle	Result	Reference
Rabbit	Japanese albino	M + F	Oral	96.2%; corn oil	LD_{50} M: 675 mg/kg bw F: 510 mg/kg bw	Hara & Suzuki (1980)
Dog	Beagle	M + F	Oral	96.2% (adjusted to 100% for dose calculation); gelatine capsule	LD_{50} > 1000 mg/kg bw	Pence et al. (1979)
Rat	Sprague-Dawley	M + F	Dermal	97.0%; corn oil	LD_{50} M: 1600 mg/kg bw F: 870 mg/kg bw	Kohda (1979b)
Rat	Sprague-Dawley	M + F	Dermal	93.8%; corn oil	LD_{50} > 5000 mg/kg bw	Omodaka et al. (1986b)
Rat	Sprague-Dawley	M + F	Dermal	92.7%; no vehicle	LD_{50} > 2000 mg/kg bw	Asano (2011b)
Rabbit	New Zealand White	M + F	Dermal	Technical grade; no vehicle	LD_{50} > 2000 mg/kg bw	Marroquin et al. (1981)
Mouse	ICR	M + F	Inhalation (whole body)	97% (formulated at 200 g/l in distilled water)	LC_{50} (3 h) M: 100 mg/m^3 F: 43 mg/m^3 NOEC 4.5 mg/m^3	Kohda et al. (1976)
Mouse	Swiss Webster	M + F	Inhalation (whole body)	94.5%	LC_{50} > 0.009–0.011 mg/l	Bruce et al. (1986)
Rat	Sprague-Dawley	M + F	Inhalation (whole body)	97% (formulated at 200 g/l in distilled water)	LC_{50} (3 h) > 96 mg/m^3 NOEC 12 mg/m^3	Kohda et al. (1976)
Rat	Sprague-Dawley	M + F	Inhalation (whole body)	94.5%	LC_{50} > 0.009–0.011 mg/l	Bruce et al. (1986)
Rat	Sprague-Dawley	M + F	Inhalation (mist, nose only)	92.7%	LC_{50} 556–1340 mg/m^3	Asano (2011c)
Mouse	dd	M + F	Subcutaneous	97.0%; corn oil	LD_{50} M: 1350 mg/kg bw F: 900 mg/kg bw	Kohda & Kadota (1976)
Rat	Sprague-Dawley	M + F	Subcutaneous	97.0%; corn oil	LD_{50} M: 1410 mg/kg bw F: 900 mg/kg bw	Kohda & Kadota (1976)
Mouse	dd	M + F	Intraperitoneal	97.0%; corn oil	LD_{50} M: 230 mg/kg bw F: 210 mg/kg bw	Kohda & Kadota (1976)
Rat	Sprague-Dawley	M + F	Intraperitoneal	97.0%; corn oil	LD_{50} M: 225 mg/kg bw F: 180 mg/kg bw	Kohda & Kadota (1976)

Table 5 (continued)

Species	Strain	Sex	Route	Purity; vehicle	Result	Reference
Mouse	Swiss Webster	M	Intravenous	Not given; glycerol formal	LD_{50} 4.5 mg/kg bw	Summitt & Albert (1980)
Rabbit	Albino	M	Skin irritation	90.2%	Non-irritating	Matsubara et al. (1979)
Rabbit	New Zealand White	M	Skin irritation	93.0%	Slightly irritating	Young-Ha (2011a)
Rabbit	Albino	M	Eye irritation	90.2%	Mildly irritating	Matsubara et al. (1979)
Rabbit	New Zealand White	M	Eye irritation	93.0%	Minimally irritating	Young-Ha (2011b)
Guinea-pig	Hartley	M	Skin sensitization	97%; corn oil	Non-sensitizing	Okuno, Kadota & Miyamoto (1975)
Guinea-pig	Hartley	M	Skin sensitization (Buehler method)	88.1%; acetone	Non-sensitizing	Suzuki & Miyamoto(1981)
Guinea-pig	Hartley	M	Skin sensitization (Buehler method)	93.0%; acetone	Non-sensitizing	Kawabe (2011)
Impurity						
Mouse	dd	M + F	Oral	TMPA-AH > 99%	LD_{50} M: 1450 mg/kg bw F: 1880 mg/kg bw	Misaki et al. (1981)

F, female; LC_{50}, median lethal concentration; LD_{50}, median lethal dose; M, male; NOEC, no-observed-effect concentration; TMPA-AH, 2,2,3,3-tetramethylcyclopropanecarboxylic anhydride

The impurity 2,2,3,3-tetramethylcyclopropanecarboxylic anhydride (purity > 99%) was shown to possess significantly lower toxicity, as the LD_{50} values were 1450 mg/kg bw for male mice and 1880 mg/kg bw for female mice. Deaths occurred at 1000 mg/kg bw and above. The toxic symptoms, such as decreased spontaneous activity, ataxia, limb paralysis, irregular respiration, hyperpnoea followed by dyspnoea, piloerection and urinary incontinence, were observed at 500 mg/kg bw and above in both sexes. These symptoms developed 1–2 hours after treatment and disappeared within 3–5 days (Misaki et al., 1981).

(b) Dermal and ocular irritation

The potential of fenpropathrin to irritate the skin and eyes (see Table 5) was evaluated in rabbits. Fenpropathrin did not cause any irritation to the skin in a first study (Matsubara et al., 1979). In a second study, transient, barely perceptible erythema (degree 1) was observed; no sign of irritation was present in any of the animals 7 days after completion of exposure (Young-Ha, 2011a).

Transient redness and chemosis, disappearing by 72 hours, were observed following instillation into the eye in a first study (Matsubara et al., 1979). In a second study, initial slight redness and chemosis (degree 1) disappeared within 24 hours (Young-Ha, 2011b).

(c) Dermal sensitization

The sensitization potential of fenpropathrin (see Table 5) was evaluated in guinea-pigs in three studies, one of which used intradermal repeated inductions, another used an experimental design similar to the Buehler method and the more recent Buehler study used undiluted technical-grade fenpropathrin for both the three inductions and the challenge applications. Fenpropathrin did not show any sensitizing potential in any of these studies (Okuno, Kadota & Miyamoto, 1975; Suzuki & Miyamoto, 1981; Kawabe, 2011).

2.2 Short-term studies of toxicity

The toxicity of fenpropathrin following short-term exposure was evaluated in mice, rats and dogs. Apart from very old studies and published papers, all the studies were in compliance with modern EU guidelines and either in compliance with GLP or, where pre-1993, conducted and reported to an adequate standard.

(a) Oral administration

Mice

In a first 4-week range-finding study for a carcinogenicity study, groups of eight male and eight female CD-1 mice received fenpropathrin (purity not indicated) in their diet at a concentration of 0, 100, 200 or 300 ppm (equal to 0, 12.1, 26.9 and 40.0 mg/kg bw per day for males and 0, 14.7, 29.2 and 44.7 mg/kg bw per day for females, respectively). Stability and homogeneity of the diets were assessed. Fresh diets were prepared weekly. All animals were observed twice daily for clinical signs and mortality. Body weights were recorded prior to treatment and then weekly thereafter. Feed consumption was measured weekly. All animals were necropsied after 4 weeks of treatment and examined for gross pathology. Absolute and relative organ weights were calculated, and a complete list of tissues/organs was preserved, but not processed further.

In view of the absence of any toxic findings in this study, doses of 500, 1000 and 1500 ppm were chosen for a subsequent range-finding study (Colley et al., 1981a).

In a second 4-week range-finding study, groups of eight male and eight female CD-1 mice received fenpropathrin (purity not indicated) in their diet at a concentration of 0, 500, 1000 or 1500 ppm (equal to 0, 63, 123 and 204 mg/kg bw per day for males and 0, 69, 142 and 216 mg/kg bw per day for females, respectively). Mice were observed twice daily for mortality and clinical signs. Body weights and feed consumption were recorded weekly. Water consumption was visually monitored daily. After 4 weeks of treatment, surviving animals were killed and necropsied. Absolute and relative organ weights were calculated for adrenals, brain, heart, kidney, liver, lung, spleen, testes and ovaries, and a complete list of tissues/organs was preserved, but not processed further.

Three males and two females receiving 1500 ppm were found dead during the 1st day of treatment. An increased incidence of piloerection was noted in the majority of males and females treated at 1500 ppm and in two males at 1000 ppm. On occasion, this sign was associated with hunched posture, lethargy, body tremors and dark discoloration of the eyes for one or two males receiving 1500 ppm and with dark discoloration of the eyes for one male at 1000 ppm. Pallor of the extremities was also noted in two males and two females treated at 1500 ppm and in one female treated at 1000 ppm. Body weight gain was reduced in males receiving 1000 or 1500 ppm. Although feed consumption was not affected by treatment, impaired efficiency of feed utilization was noted for males treated at 1000 ppm and for animals of both sexes at 1500 ppm. Necropsy of decedent mice and of mice sacrificed at termination of the study did not reveal any treatment-related findings. Analysis of organ weight data revealed a dose-related increase in liver weights of male mice, not attaining statistical significance for those treated at 500 ppm. Relative kidney weight was also increased in males at 1500 ppm. These differences were not apparent in females.

The NOAEL was 500 ppm (equal to 63 mg/kg bw per day), based on decreases in body weight gain in males, decreases in feed efficiency in males and clinical signs seen at 1000 ppm (equal to 123 mg/kg bw per day). The dose of 1000 ppm was considered suitable for a long-term carcinogenicity study in mice (Colley et al., 1981b).

Although a 2-year carcinogenicity study in CD-1 mice (52 of each sex per dose) with fenpropathrin (purity not indicated) was started with dose levels of 0, 40, 200 and 1000 ppm, as a result of the high mortality in mice receiving 200 and 1000 ppm, the study was terminated after 13 weeks of treatment. Achieved doses until week 13 in that study were 0, 4.9, 24.7 and 130 mg/kg bw per day for male mice and 0, 5.7, 27.8 and 139 mg/kg bw per day for female mice, respectively. At termination, laboratory investigations were not performed. Macroscopic examination was performed on all animals that died prematurely or were killed and on 20 males and 20 females from each group. Histopathological examination was limited to mice that died prematurely or were killed during the first 11 weeks of the treatment.

Occasional body tremors were noted for a few males receiving 1000 ppm from week 1 onwards and for one male receiving 200 ppm in week 2. Nineteen males and eight females receiving 1000 ppm, four males receiving 200 ppm, one male and one female receiving 40 ppm and two control males were found dead. One additional male receiving 1000 ppm was killed due to poor conditions. The cause of death for these animals was not established. The clinical signs (tremors) were mostly observed in moribund animals. Slightly higher feed consumption and body weight gain were noted for males receiving 200 and 1000 ppm. Slightly higher liver weights were recorded for animals treated at 1000 ppm. No treatment-related morphological changes were detected at histopathological examination of decedent animals (Colley et al., 1982c).

Rats

In a 25-day toxicity study, groups of six male and six female Sprague-Dawley rats received fenpropathrin (purity not indicated) in their diet at 0 or 900 ppm (equivalent to 0 and 90 mg/kg bw per day) for 25 days. Daily observations on the general health and behaviour of each animal were made. Following gross pathological examination, sections of sciatic nerve were stained by the cresyl violet–luxol fast blue method for myelin and by the Glees and Marsland method for axons and then examined histologically.

Fine tremors appeared in treated male rats on day 2; by day 12, the tremors were violent, with erratic jumping in three males. One male rat was found dead on each of days 16 and 20. On day 25, the remaining four male rats still exhibiting tremors were sacrificed. In the females treated for 1 day, one rat was found dead, and three out of the five remaining showed tremors. By day 3, all five exhibited tremors, one rat showed erratic jumping and one rat was moribund and sacrificed. By day 5, all treated females were dead or sacrificed. Swelling and disintegration of nerve axons, in some cases apparently involving almost every fibre, were present in all except 1 of the 12 rats treated at 900 ppm, even in those whose survival time was very short. No definitive myelin lesion was seen. The nerves from the control animals were normal (Stevenson et al., 1976).

In a 4-week range-finding study, groups of 10 male and 10 female Sprague-Dawley rats received fenpropathrin (purity not indicated) in their diet at a concentration of 0, 1, 5, 50 or 500 ppm (equal to 0, 0.08, 0.40, 4.04 and 38.4 mg/kg bw per day for males and 0, 0.08, 0.41, 4.40 and 42.8 mg/kg bw per day for females, respectively). Fresh diets were prepared weekly. Stability, homogeneity and dietary concentrations were evaluated periodically. All animals were examined twice daily for mortality and clinical signs. Body weights and feed consumption were recorded weekly. Ophthalmic examinations were performed on all rats of the control and high-dose groups before treatment commenced and during week 4. Blood samples were taken from fasted animals of the control and high-dose groups after 4 weeks for haematology and clinical chemistry. All animals were necropsied at the end of the study and examined for gross pathology. Absolute and relative

organ weights were determined. At terminal sacrifice, a piece of liver was taken and frozen immediately (approximately −70 °C) in a bath of hexane immersed in a bath of acetone containing solid carbon dioxide. A complete list of tissues and organs was preserved in buffered 10% formalin (Davidson's fluid for the eyes). Tissues examined microscopically (liver, kidney, spleen, heart, lungs, tibial and sciatic nerves from all animals of the control and high-dose groups) were stained with haematoxylin and eosin. In addition, frozen sections of liver fixed in buffered formalin were stained with Oil Red O. Fresh frozen sections of liver were also stained with periodic acid–Schiff reagent to demonstrate glycogen. Sections of kidney were stained with Oil Red O or periodic acid–Schiff reagent at the discretion of the pathologist. Nerve tissue was stained with luxol fast blue (Kluver and Borrera method) and silver nitrate (Glees and Marsland method).

There was no mortality or clinical signs. Treatment had no effect on body weight, body weight gain, feed consumption or efficiency of feed utilization. No abnormality was noted at ophthalmic examinations. Haematology did not reveal any difference from controls. At week 4, marginally higher group mean creatinine and alanine aminotransferase (ALT) levels were recorded in males treated at 500 ppm; however, as all individual values were within acceptable limits, these changes were not considered of biological significance. No treatment-related findings were detected at necropsy. Slightly higher relative spleen weight and marginally higher liver weights, not attaining statistical significance, were noted in males treated at 500 ppm. No treatment-related findings were noted at microscopic examination.

The NOAEL was 500 ppm (equal to 38.4 mg/kg bw per day in males), the highest dose tested. A further range-finding study was considered appropriate for setting dose levels for the combined toxicity/carcinogenicity study (Colley et al., 1982a).

In another 4-week range-finding study, groups of 10 male and 10 female Sprague-Dawley rats received fenpropathrin (purity not indicated) in their diet at a concentration of 0, 500, 700, 1000 or 1500 ppm. The mean compound intakes in males were 44 and 65 mg/kg bw per day at 500 and 700 ppm, respectively. The mean compound intakes in females were 51 and 85 mg/kg bw per day at 500 and 700 ppm, respectively. Because of a high incidence of mortality or severe clinical signs, all surviving animals treated at 1000 and 1500 ppm were terminated on days 4 and 3, respectively. Fresh diets were prepared weekly. Stability, homogeneity and concentrations of diets were confirmed analytically. All animals were examined twice daily for mortality and clinical signs. Body weights and feed consumption were recorded weekly. Ophthalmic examinations were performed on all rats of the control and high-dose groups before treatment commenced and during week 4. Samples were taken from all fasted surviving animals after 4 weeks for haematology and clinical chemistry. All animals were necropsied at the end of the study and examined for gross pathology. Absolute and relative organ weights were determined. A complete selection of tissues and organs was preserved, but not processed further.

In the morning of day 2, one male and three females treated at 1500 ppm and one male treated at 700 ppm were found dead. Eight of the remaining nine males and five of the remaining seven females treated at 1500 ppm showed marked body tremors and involuntary movements. Similar but less marked signs were noted in most females treated at 1000 ppm, in one or more males receiving 700 or 1000 ppm and in all females treated at 700 ppm. The severity of the signs was considered to be associated with nocturnal feeding, because they became less severe during the day, although an additional female treated at 1500 ppm died and another one was killed due to marked clinical signs. The following day, further deaths were recorded in male and female rats treated at 1500 ppm; in view of the severity of the clinical signs, the surviving rats of this dose group were terminated. Overnight mortalities were also recorded among rats of all treated groups. Body tremors were noted in most males and a few females treated at 1000 ppm, and less marked signs were noted in a few rats receiving 700 ppm. On day 4, following further overnight mortalities and the presence of severe clinical signs in rats treated at 1000 ppm, all surviving rats of this dose group were terminated. From day 4 onwards, one male and three females at 500 ppm and two males and four females at 700 ppm

were found dead. During this period, body tremors were recorded on isolated occasions in rats treated at 700 ppm and in a few females at 500 ppm. One female at 500 ppm and one male and two females at 700 ppm were killed due to severe clinical signs or moribund conditions. General signs of hypersensitivity were noted in rats of both sexes treated at 500 or 700 ppm. Slightly lower body weight gain was noted in males receiving 700 ppm and in females at 500 ppm. A more marked reduction in body weight gain was noted in the single surviving female at 700 ppm. This was associated with slightly lower feed intake in males, but not in females, whereas efficiency of feed utilization appeared reduced, compared with the controls, in females at 700 ppm only. Haematological examinations at week 4 revealed increases in red blood cell and neutrophil counts and increased haemoglobin concentration with reduced mean corpuscular volumes (not attaining statistical significance) in males at 700 ppm. Slightly lower platelet counts were noted in males at 500 and 700 ppm. Marginally lower total white cell and neutrophil counts were recorded in most females treated at 500 ppm, with a similar effect also noted for the surviving female at 700 ppm. Clinical chemistry revealed lower calcium levels in males at 700 ppm and marginally lower globulin levels in males at 500 and 700 ppm. Marginally lower total protein and albumin levels were recorded in females treated at 500 ppm and in the surviving female at 700 ppm. No changes were noted at ophthalmic examination.

Necropsy of decedent animals, sacrificed animals or animals at termination did not show any treatment-related findings. Relative kidney weight was increased in females at 500 ppm and in males at 700 ppm (not attaining statistical significance).

A NOAEL was not established in this study. The lowest-observed-adverse-effect level (LOAEL) was 500 ppm (equal to 44 mg/kg bw per day) (Colley et al., 1982b).

The difference in results at 500 ppm between the two 4-week oral studies is considered to be related to the achieved test compound intake. This was maximal during the first 2 weeks of dosing and was 45.4 mg/kg bw per day in males and 47.5 mg/kg bw per day in females in the first study, whereas it was 53.13 mg/kg bw per day in males and 59.69 mg/kg bw per day in females in the second study. Results suggest a steep dose–response relationship, with the NOAEL being around 50 mg/kg bw per day in the rat (Heywood, 1982).

In a first 13-week study, groups of 12 male and 12 female Carworth Farm E rats received fenpropathrin (purity 96%) in powdered diets at a concentration of 0, 2, 10, 50 or 250 ppm (equivalent to 0, 0.1, 0.50, 2.5 and 12.5 mg/kg bw per day, respectively); the control group consisted of 24 male and 24 female rats. Daily observations were made on the general health and behaviour of each animal. Body weights and feed intakes were recorded weekly. The animals were necropsied after 13 weeks. Following gross pathological examination, major viscera were weighed, and microscopic examination of a selection of organs from all animals in the control, 50 and 250 ppm groups was carried out. Terminal blood samples were taken by cardiac puncture for haematological and clinical chemistry examinations.

No clinical signs or behavioural changes were noted during the study. In male rats, there were increases in body weight gain at weeks 1, 2 and 3 at all dose levels and at week 4 in those treated at 50 and 250 ppm only. This effect was seen in females only in week 1 at 10, 50 or 250 ppm. Feed intake was comparable among the treated and control groups. A slightly lower relative liver weight was recorded in rats of both sexes treated at 10 ppm, but not in those treated at higher dose levels. Absolute and relative spleen weights were increased in males at 250 ppm; this was the only change concerning organ weights and may present a borderline effect. No treatment-related changes were noted in haematological or clinical chemistry parameters, and no findings related to treatment were noted at histopathology (Hend & Butterworth, 1975).

In a second 13-week study, groups of 12 male and 12 female Sprague-Dawley rats received fenpropathrin (purity 97%) in their diet at a concentration of 0, 3, 30, 100, 300 or 600 ppm (equivalent to 0, 0.15, 1.5, 5, 15 and 30 mg/kg bw per day); the control group consisted of 24 male and 24 female rats. The 600 ppm treatment level was added to the test design late and thus was handled separately from the other treatments and was not included in the block randomization. However, the results for this treatment level were compared with the controls in the main test. Daily observations on the general health and behaviour of each animal were made. Body weights and feed intakes were recorded weekly. The animals were necropsied after 13 weeks. Terminal blood samples were taken by cardiac puncture for haematological and clinical chemistry examinations. Following gross pathological examination, major viscera were weighed, and a selection of organs from all animals in the control, 100, 300 and 600 ppm groups was examined microscopically.

General health and body weight gain were unaffected in all animals except those treated at 600 ppm. Tremors occurred in 1 out of 12 males and 9 out of 12 females. The tremors appeared after 5 weeks of treatment, but disappeared completely later in the study (by week 11). Significant ($P < 0.01$) decreases in body weight (8–14%) were seen in females receiving 600 ppm throughout the study. High-dose males had significant reductions in body weight from week 1 to week 5. Body weights for lower treatment groups were comparable with those of the controls. Examination of haematological parameters revealed decreases in haemoglobin concentration and mean erythrocyte volume and an increase in prothrombin time in males treated at 600 ppm. Clinical chemistry revealed an increase in plasma alkaline phosphatase activity in rats of both sexes receiving 600 ppm (33% males; 42% females) and a slight increase in plasma potassium in males only. Increases in relative kidney weight in males (7.2%) and relative brain weight in females (6.2%) treated at 600 ppm were observed. No microscopic findings were noted at histopathology.

The NOAEL was considered to be 300 ppm (equivalent to 15 mg/kg bw per day), based on clinical signs of tremors, body weight reductions, decreased blood clotting time in females and possibly increased alkaline phosphatase levels (both sexes) at 600 ppm (equivalent to 30 mg/kg bw per day) (Hend & Butterworth, 1976).

In a third 13-week toxicity study, groups of 12 male and 12 female Sprague-Dawley (Crj:CD) rats received fenpropathrin (purity 93.1%) in pulverized diet at a concentration of 0, 15, 50, 150, 450 or 600 ppm (equal to 0, 0.72, 2.49, 7.22, 21.3 and 28.8 mg/kg bw per day for males and 0, 0.82, 2.82, 8.18, 25.2 and 36.1 mg/kg bw per day for females, respectively). Stability, homogeneity and concentrations of the prepared diets were confirmed analytically. All animals were observed daily for clinical signs, and detailed clinical examinations were performed at least weekly. Body weights and feed consumption were recorded weekly. Ophthalmic examinations were carried out on all animals at initiation of treatment and on all surviving animals of the control and 600 ppm groups during week 13. Haematology, clinical chemistry and urine analysis evaluations were performed on all animals during week 13 or at termination. Gross necropsy and histopathology were conducted on all animals; absolute and relative organ weights were determined.

A single female treated at 600 ppm died on day 46, but it was not possible to determine the cause of death. No clinical signs were observed during the study in any of the treated or control animals. Body weights of rats treated at 600 ppm were significantly reduced, compared with controls, during the 1st week of treatment. Thereafter, body weights in males were just slightly, but not significantly, lower than those of controls, whereas body weights of females remained significantly lower than control values for the rest of the study, with terminal body weights 10% lower than control values. Females treated at 450 ppm also showed slightly lower body weights when compared with controls during the first 2 weeks of treatment, with statistical significance attained on week 2 only. At 150 ppm, the mean body weight of males at week 13 was significantly higher than that of controls. Effects on body weight were in general accompanied by reductions in feed consumption, and calculation of efficiency of feed conversion showed lower efficiency for females at 600 ppm for the entire treatment period, with a few exceptions in weeks 3, 4 and 6. No treatment-related observations

were noted at ophthalmic examinations. At urine analysis, increases in urine protein and specific gravity were noted in males treated at 450 and 600 ppm. As there were no changes in total protein or blood urea nitrogen at clinical chemistry and no abnormal findings in kidney at histopathology, the increases in urine protein and specific gravity were not considered to be toxicologically significant. No differences from controls were noted at haematology, whereas clinical chemistry revealed an elevated activity of alkaline phosphatase in females treated at 600 ppm. No treatment-related findings were observed at necropsy; the decedent female at 600 ppm had dark-coloured and oedematous lungs. Analysis of organ weight data showed an increase of relative brain weight in females at 600 ppm, an increase in relative kidney weight in females at 450 ppm and a decrease in absolute testes weight in males at 15, 50 and 150 ppm, with relative testes weight also decreased at 150 ppm. As the changes in relative brain weight were accompanied by a reduction in body weight, these changes were not considered to be indicative of a direct effect of treatment. As the changes in relative kidney weight and testes weights were not observed at the highest dose level of 600 ppm, these changes were not considered treatment related. The incidence of hepatic microgranulomas was significantly increased in males treated at 450 ppm, but not in males at 600 ppm, and decreased in females at 15, 50, 450 and 600 ppm. One female treated at 450 ppm had a nephroblastoma. Pulmonary congestion and oedema were microscopically observed in the decedent female. As the histopathological findings described above were usually observed in young rats or were not observed at the highest dose level of 600 ppm, they were not considered treatment related.

The NOAEL was 450 ppm (equal to 21.3 mg/kg bw per day), based on decreased body weight gain, decreased feed consumption and slightly increased alkaline phosphatase activity seen at 600 ppm (equal to 28.8 mg/kg bw per day) (Yosida et al., 1986).

Dogs

In a first range-finding study, one male and one female purebred Beagle dog received fenpropathrin (purity 96.2%, adjusted to 100% for dosage calculation) in their diet at a concentration of 4000 ppm (equivalent to 100 mg/kg bw per day) for 4 days. They were then returned to control feed because the animals were eating only very small quantities of the treated diet and suffered severe emesis. After 3 days on control feed, the dogs were given a diet containing fenpropathrin at 2000 ppm for the remaining 8 days of the study. All dogs were observed for mortality and clinical signs twice daily. Feed consumption was determined on days 5, 8 and 15. On day 15, all dogs were anaesthetized with thiamylal sodium, sacrificed by exsanguination following an overnight fast and examined macroscopically.

Severe emesis and mucoid or blood mixed with mucoid faeces were noted in the two treated dogs receiving 4000 ppm in the diet. When returned to control diet on day 5, the dogs appeared normal except for one incident of dried food emesis. After day 8, the dogs again received a treated diet at 2000 ppm, and observations included tremors and absence of faeces. On day 15, both dogs were observed to have low posture. The female dog additionally had slight tremors, moderate ataxia and decreased locomotor activity. Both animals were sacrificed the following day. Body weight loss between 1 and 2 kg was observed during the study. This was associated with a marked reduction in feed intake. At necropsy, one dog had no findings, whereas the other one had a kidney with cut surface reddened (Pence et al., 1979).

In a 13-week study, groups of six male and six female Beagle dogs received fenpropathrin (purity 96.2%, adjusted to 100% for dosage calculation) in their diet at a concentration of 0, 250, 500 or 1000/750 ppm (1000 ppm for weeks 1–3, 750 ppm for weeks 4–13). Mean substance intakes were 0, 7.4, 15.5 and 24 mg/kg bw per day for males and 0, 9.6, 15.9 and 28.7 mg/kg bw per day for females, respectively. Fresh diets were prepared weekly. All animals were observed for clinical signs and mortality daily for the first 2 weeks and then weekly for the remainder of the period. Criteria evaluated included survival, appearance and behaviour, body weight (weekly), feed consumption (weekly), clinical laboratory studies (weeks 0, 4, 8 and 13), urine analysis (weeks 0 and 13),

ophthalmic examinations (weeks 0 and 13), terminal body weight and organ weight data, and gross and microscopic pathology.

There were no definite dose-related effects seen in the clinical chemistry, urine analysis, gross necropsy, organ weights, histopathology or ophthalmological analyses. One high-dose male was sacrificed moribund during week 3, showing tremors and decreased locomotor activity. Clinical signs of toxicity at all treatment levels included soft and mucoid stools and/or diarrhoea, emesis, tremors, ataxia and sometimes lethargy, panting and salivation. The tremors and ataxia were generally first observed during the 2nd week of treatment and were of such severity in the high-dose dogs that the dose level for this group was lowered to 750 ppm at the beginning of week 4. The frequency of the observed clinical signs decreased after week 5 in all treated groups. Slight differences in body weight gain were found in the mid-level female group (6% decrease relative to controls) and in both sexes in the high-dose group (6% decrease in males, 7% decrease in females, relative to controls). The mean haematocrit, haemoglobin and erythrocyte count values were decreased in dogs of both sexes treated at the highest concentration at weeks 4, 8 and 13. The LOAEL was 250 ppm (equal to 7.4 mg/kg bw per day), based on effects on the gastrointestinal system, tremors and nervousness. A NOAEL was not established in this study (Pence et al., 1980a,b).

In a 1-year toxicity study, groups of four male and four female Beagle dogs received fenpropathrin (purity 92.5%, adjusted to 100% for dosage calculation) in their diet at a concentration of 0, 100, 250 or 750 ppm (equal to 0, 3.1, 8.2 and 24.4 mg/kg bw per day for males and 0, 3.1, 7.7 and 24.7 mg/kg bw per day for females, respectively). The homogeneity and stability of the diets were assessed by analytical methods. Fresh diets were prepared weekly, and concentrations were confirmed periodically. Haematology, blood biochemistry and urine analysis evaluations were conducted for all animals prior to treatment initiation and at weeks 13, 26 and 52. Ophthalmic examinations were performed on all animals prior to initiation and at termination of treatment. Gross necropsy and histopathological evaluations were performed for all animals at study termination, including myelin- and axon-specific stains (Klüver-Barrera luxol fast blue stain for myelin and Hirano-Zimmerman silver stain for nerves); organ weights were recorded for all animals at termination.

One high-dose male was found dead during week 32. Prior to death, the animal was languid and thin and exhibited ataxia, tremors and polypnoea (excessive respiration). There were numerous gross findings (including soft brain, enlarged and congested liver, enlarged lymph nodes, ulcerations and erosions of the oral cavity, perforations of the tongue and multiple skin sores) and histopathological findings (including adrenal congestion and haemorrhage, pulmonary oedema and congestion, liver, kidney and stomach congestion, decreased spermatogenesis, prostate atrophy, thymus atrophy and multiple lesions of the oral cavity). There were, however, no other deaths and no gross pathological or histopathological lesions in any other treatment group. Clinical signs included tremors (750 ppm during week 1 to termination; 250 ppm starting at week 6 and sporadically thereafter); ataxia (750 ppm starting at week 2 and consistently observed from week 8 to week 32 and sporadically observed from week 33 to week 53); and languid appearance (750 ppm, weeks 7 through 48). Mean body weight of high-dose dogs was reduced (< 11% in males and 20% in females). No effect was seen on body weight in the lower treatment groups. Feed consumption, ophthalmic examinations, haematology, clinical chemistry and urine analysis as well as gross and microscopic pathology and organ weight data did not reveal any treatment-related findings.

The NOAEL was 100 ppm (equal to 3.1 mg/kg bw per day), based on occurrence of tremors in dogs treated at 250 ppm (equal to 7.7 mg/kg bw per day) (Pence et al., 1984).

(b) Dermal application

Rats

In a 21-day dermal toxicity study, fenpropathrin (purity 92.0%) was applied to the shaved intact skin of 10 Sprague-Dawley rats of each sex per dose at a dose level of 0, 50, 200 or 1000 mg/kg

bw per day for 6 hours/day, 7 days/week, for 21 days. At the end of the 6-hour exposure period, the dressings were removed, the residual test article was wiped from the application area with gauze immersed in lukewarm water and the application area was dried with tissue paper. The animals were restrained with neck collars throughout the administration period. The animals were examined for clinical signs and mortality twice a day. Animals were weighed prior to initiation of dosing, on days 1, 4, 8, 11, 15, 18 and 21 and at study termination. The eyes of all animals were examined on the day prior to initiation of treatment, and rats in the control and high-dose groups were examined again during week 3 (day 19). After the end of the administration period, all of the animals were fasted for approximately 18–24 hours, and blood was collected for evaluation of clinical chemistry and haematological parameters. Selected organs were weighed, and a complete macroscopic examination was conducted. Selected tissues and organs from the control and high-dose animals were also examined histopathologically.

All animals survived until scheduled termination, and there were no adverse effects of treatment on clinical observations, body weight, feed consumption, ophthalmoscopic examinations, haematology, organ weights or gross pathology in either sex. A transient change in feed consumption was noted in females on day 1; however, there was no effect on the overall body weight or body weight gain. Triglycerides were increased by 33% over controls in the 200 and 1000 mg/kg bw per day females. However, there were no other differences in clinical chemistry, indicating an effect on lipid metabolism. Similarly, there were no effects of treatment on liver weights and no treatment-related macroscopic or microscopic findings in the liver. Thus, the increased triglycerides were considered of equivocal toxicological significance. Examination of the treated application site indicated squamous cell hyperplasia and mononuclear cell infiltration in the dermis and crust. However, all of these lesions were slight in severity and were not accompanied by other signs of dermal irritation (e.g. oedema, erythema, scabbing or necrosis), and therefore they were not considered adverse.

The NOAEL was 1000 mg/kg bw per day, the highest dose tested (Furukawa, 2006; Ohta, 2006).

Rabbits

In a 21-day dermal toxicity study in rabbits, groups of five male and five female New Zealand White rabbits were dosed 5 days/week for 3 weeks (15 doses) with fenpropathrin (purity 91.4%) on abraded or unabraded skin at a dose of 0, 500, 1200 or 3000 mg/kg bw per day. The test article was premoistened with normal saline and evenly distributed over the rabbits' backs. The high-dose animals were dosed over 15–20% of their total skin surface. The dosing sites were occluded, and the rabbits were fitted with collars to prevent ingestion of the test article. The skin application site was washed with tap water after 6 hours of exposure. The animals were examined for clinical signs and mortality twice a day. Body weight was determined before the start of the administration period in order to randomize the animals and twice weekly thereafter. Skin irritation was scored daily. The blood sample from each treated rabbit was analysed for clinical chemistry and haematological parameters. Selected organs were weighed, and a complete macroscopic examination was conducted. Selected tissues and organs were also examined histopathologically.

A low-dose rabbit died of unknown causes. Many high-dose rabbits had doubtful or barely perceptible erythema and oedema. There were no dose-related effects on mortality, clinical signs, body weight, feed consumption, haematology or clinical chemistry parameters, gross pathology or organ weights. No histopathological findings were noted in the liver or kidneys. Trace or mild "inflammatory cell infiltrate" in the intact and abraded skin, which was noted in males and females receiving all doses, including the controls, was attributed by the study authors to the test material.

The NOAEL was 3000 mg/kg bw per day, the highest dose tested (Riley et al., 1982).

(c) Exposure by inhalation

No studies are available.

2.3 *Long-term studies of toxicity and carcinogenicity*

All studies were certified as complying with GLP.

Mice

In a carcinogenicity study in mice, groups of 52 CD-1 mice of each sex per dose received fenpropathrin (purity 91.04% and 92.5%) in their diet at a concentration of 0, 40, 150 or 600 ppm for 104 weeks (equal to 0, 3.9, 13.7 and 56.0 mg/kg bw per day for males and 0, 4.2, 16.2 and 65.2 mg/kg bw per day for females, respectively). Each group also contained 40 satellite animals of each sex for interim sacrifice after 26, 52 and 78 weeks of treatment. Stability, homogeneity and dietary concentrations were confirmed analytically. All animals were observed for clinical signs and mortality twice a day during weekdays and once a day during weekends. Body weight and feed consumption were recorded weekly. Water intake was measured daily for 5 days during weeks 24, 50 and 76 and prior to termination. Haematological investigations were performed on 10 animals of each sex per group from the satellite groups at weeks 25, 53 and 79 (all surviving satellite animals) and on all surviving mice at termination. Clinical chemistry investigations were similarly performed at weeks 24, 53 and 79 and at termination. Urine analysis was performed at weeks 26, 51 and 77 and at termination. Interim sacrifices occurred at weeks 26 and 52 (10 animals of each sex per group) and week 78 (all surviving satellite animals). Terminal sacrifice was performed at completion of 104 weeks of treatment. Pathology procedures included necropsy, organ weights and histopathological examination of a complete list of tissues and organs.

Although, in the earlier part of the study, a marginal increase in the number of females receiving 600 ppm showing hyperactivity was noted when compared with controls, there was no evidence of any effect on the incidence of hyperactivity reported after 78 weeks of treatment or beyond. Hyperactivity was primarily observed in animals in moribund condition. There was no evidence of a treatment-related effect on survival. In order of ascending doses, total mortalities at study termination, out of the 52 main mice of each sex per group, were 24, 31, 23 and 24 for males and 23, 23, 33 and 28 for females. No effects on body weight, body weight gain, feed consumption, feed efficiency, urine analysis, macroscopic pathology or organ weights were reported. Changes in haematological parameters at 600 ppm included slight decreases in haemoglobin and mean corpuscular haemoglobin concentration (MCHC) in males and an increase in MCHC in females at week 25. Because these changes were small, occurring only on some occasions and occurring in one sex only, they are not considered to be toxicologically significant. Sporadic changes in clinical chemistry parameters noted at week 53 included a slight decrease in urea nitrogen in males at 600 ppm and in all groups of treated females. Males treated at 600 ppm also had significantly increased ALT and aspartate aminotransferase (AST) levels. Higher ALT and AST levels were also recorded among females receiving 150 or 600 ppm. In view of the absence of these differences at week 79 or at termination, these changes were not considered to be of toxicological significance. Brain weights of mice of both sexes treated at 600 ppm were higher than those of controls at the interim kill on week 27 and at termination. It was also noted that at week 27, brain weights of all treated female groups were higher than those of controls, but without any clear evidence of a treatment-related trend. Increased brain weights were again recorded for females only at week 78, whereas no effect was recorded at the interim sacrifice on week 53. Kidney weights of females treated at 600 ppm were higher than those of controls at the terminal sacrifice. Higher kidney weights were also recorded for females of this group at weeks 27 and 79, although not attaining statistical significance. In males treated at 600 ppm, kidney weights were elevated at week 53. The changes in brain weights and kidney weights were not considered to be of toxicological significance, because the changes were small in magnitude and lacked a clear dose–response relationship and/or statistical significance. No treatment-related effects were noted on the incidence of any of the non-neoplastic findings detected at histopathology.

An increased incidence of lung tumours (adenoma and adenocarcinoma) was observed in males and females of treated groups. The incidences in the satellite groups (spontaneous deaths and interim sacrifice after 26, 52 and 78 weeks) were 10%, 12.5%, 18% and 2.5% for males and 7.5%,

12.5%, 7.5% and 7.5% for females at 0, 40, 150 and 600 ppm, respectively. The incidences in the main study were 12%, 23%, 33% and 31% for males and 10%, 29%, 17% and 13% for females at 0, 40, 150 and 600 ppm, respectively. The incidences did not show a clear dose–response relationship. The absence of any statistical significance between the highest group and controls and any significant trend supports the interpretation that the increased incidences in the treatment groups were not due to a tumorigenic activity of fenpropathrin. Moreover, data on the incidence of lung tumours in historical controls showed that incidences of pulmonary adenoma and adenocarcinoma in untreated animals may vary between 7% and 36%. There was no sex difference concerning lung tumours in these controls. Therefore, the results of this study gave no evidence of fenpropathrin-induced carcinogenic potential.

Fenpropathrin had no carcinogenic effects in mice. The NOAEL was the highest dose tested, 600 ppm (equal to 56.0 mg/kg bw per day in males and 65.2 mg/kg bw per day in females) (Colley et al., 1985; Colley & Gopinath, 1987). A repeat study is not necessary, because the aborted mouse carcinogenicity study (described above in section 2.2) demonstrated that at a slightly higher MTD of 1000 ppm, the test article was lethal to 38% of males and 15% of females after only 13 weeks, indicating a steep toxicity–response curve. Thus, the maximum dose used in the completed study (600 ppm) was very close to the MTD.

Rats

In a combined chronic toxicity and carcinogenicity study, groups of 24 COBS rats of each sex per dose were fed diets containing 0, 1, 5, 25, 125 or 500 ppm (equivalent to 0, 0.05, 0.25, 1.25, 6.25 and 25 mg/kg bw per day) fenpropathrin (purity 97%) for 2 years. The control group consisted of 48 rats of each sex. In addition, groups of six rats of each sex per dose received treated diets at the same concentrations for 6 or 12 months, with concurrent controls consisting of 12 male and 12 female rats. Stability, homogeneity and concentrations of the diet were confirmed analytically. Animals were examined daily for clinical signs and mortality. Rats dying or removed prior to scheduled termination were examined macroscopically. At termination, blood was collected from all animals for haematology and clinical chemistry measurements. At necropsy, all animals were examined macroscopically for any abnormalities. Selected organs were removed, weighed and examined histopathologically.

The treatment did not affect the survival, general health or behaviour of the animals or their clinical chemical or haematological parameters. The depression in body weight gain observed at 500 ppm was statistically significant in females only. Feed consumption of treated animals was comparable with that of control animals at most observation times. Absolute spleen weight was increased at 500 ppm in females of the 6-month satellite group. Relative heart weights were increased at 125 and 500 ppm in females of the 6-month satellite group. Absolute kidney weights were decreased in females at all doses in the 12-month satellite groups without showing a clear dose–response relationship. In the 2-year main study groups, no treatment-related alterations in organ weights were observed, except for relative liver weight, which was statistically significantly increased in females at 125 and 500 ppm. Gross pathological examination showed a higher incidence of white/grey foci or plaques in the lungs at 125 ppm in males at 24 months and 500 ppm in females at 6 and 24 months. In the 2-year main groups, the number of deaths attributable to renal failure in males at 500 ppm was greater than in the other groups, but no increase was observed when animals from the satellite and main study groups were combined. The histopathological neoplastic and non-neoplastic changes found were consistent with the range and severity of changes usually observed in this rat strain and did not give any evidence of carcinogenicity.

The NOAEL was 125 ppm (equivalent to 6.25 mg/kg bw per day), based on depression in body weight gain at 500 ppm (equivalent to 25 mg/kg bw per day) (Hend & Gellatly, 1979; Aitken & Rushton, 1981).

In a second combined chronic toxicity and carcinogenicity study, groups of 50 CD rats of each sex per dose received fenpropathrin (purity 91.4–92.5%) in their diets at a concentration of 0, 50,

150, 450 or 600 ppm (equal to 0, 1.9, 5.7, 17.1 and 22.7 mg/kg bw per day for males and 0, 2.4, 7.1, 21.9 and 38.8 mg/kg bw per day for females, respectively) for 2 years or more, until any one group (each sex separately) had reached a survival rate of 25%. Because of excessive mortality, females treated at 600 ppm were terminated after 52 weeks of treatment. The treated male and female rats were terminated after 104 and 113 weeks of treatment, respectively. Additional satellite groups consisting of 15 CD rats of each sex were treated at the same dose levels and used for blood sampling at intervals up to week 52 before termination after 52 weeks of treatment. Dietary stability, homogeneity and concentrations were confirmed analytically. Treated animals were observed for mortality and moribundity twice during weekdays and once on weekends. Body weights and feed consumption were recorded every week. Ophthalmic examinations were performed before treatment commenced and during weeks 6, 13, 26, 52, 78 and 104 on all control animals and the highest surviving dietary level groups. Haematology, clinical chemistry and urine analyses were conducted on 10 rats of each sex per dose from satellite group rats at weeks 25/26 and 51/52 and from main group animals at weeks 77/78, 103/104 and 107. All surviving animals from the satellite group and the main group were subjected to detailed macroscopic examination and organ weight analysis. A full spectrum of tissue samples was preserved for histopathological examination; however, only nervous tissues and liver and kidneys were processed and examined.

Clinical signs were restricted to body tremors, most prevalent in females receiving 600 ppm, but were also observed in males at the highest dose levels in the first few weeks of the study and in females at 450 ppm. Tremors were observed during the early part of the working day and essentially during weeks 1–52. Mortality increased in males and females receiving 600 ppm and in females receiving 450 ppm during the first 26 weeks of treatment; subsequently, mortality was less than that of control animals, resulting in highest survival rates in males at 600 ppm and in females at 450 ppm. Exposure of main group females receiving 600 ppm was terminated after 52 weeks. Feed consumption was comparable with that of controls except for slightly higher feed consumption reported for the high-dose females during the first 3 months of treatment. Body weight gain was reduced in females at 600 ppm, and feed utilization efficiency was marginally impaired. Ophthalmological examination did not reveal any treatment-related ocular lesions. No abnormal haematological findings were reported. No treatment-related changes in urinary parameters were observed. Changes in clinical chemistry parameters at 450 and 600 ppm included slightly reduced creatinine levels and reduced total protein levels in males. However, these alterations in clinical chemistry were of no toxicological relevance, because they were minimal or marginal changes, with no dose–response relationship and a lack of corroborative histopathological findings. Macroscopic examination revealed no findings that were treatment related, with the exception of an increased incidence of alopecia in females at 600 ppm at 52 weeks. Changes in organ weights (liver, kidney and others) were observed on some occasions, but did not show a treatment-related pattern, and histopathological examination did not reveal changes attributable to treatment. A few lymphoreticular tumours were seen in treated groups of male rats, but none in the control group. The increases observed were not dose related and were within the incidence range of the historical controls for this rat strain. There was no indication of a treatment-related increase in tumour incidence or non-neoplastic organ changes. The electron microscopic examination of tibial nerve did not reveal any treatment-related effects.

The NOAEL for males was 450 ppm (equal to 17.1 mg/kg bw per day), based on increased mortality and body tremors at 600 ppm (equal to 22.7 mg/kg bw per day). The NOAEL for females was 150 ppm (equal to 7.1 mg/kg bw per day), based on increased mortality and body tremors at 450 ppm (equal to 21.9 mg/kg bw per day) (Warren et al., 1986; Dean et al., 1987).

2.4 Genotoxicity

The results of studies of genotoxicity with fenpropathrin are summarized in Table 6. All the studies, either in vitro or in vivo, were negative. Fenpropathrin is not considered to possess any mutagenic or genotoxic potential.

Table 6. Results of studies of genotoxicity with fenpropathrin

Type of study	Organism/cell line	Dose range tested	Purity (%)	Result	Reference
In vitro					
Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and TA1538	10–1000 µg/plate	97	Negative +S9 mix (mouse, 6 strains)	Suzuki (1977)
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA1538 and *Escherichia coli* WP2*uvrA*	50–5000 µg/plate	92.5	Negative ±S9 mix	Izumozaki et al. (1984)
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA1538 and *E. coli* WP2*uvrA*	100–5000 µg/plate	92.5	Negative ±S9 mix	Kogiso et al. (1987)
Reverse mutation, mitotic crossing-over and mitotic gene conversion	*Saccharomyces cerevisiae* D7	0.001–0.1 mmol/l	92.5	Negative ±S9 mix	Hara et al. (1984)
DNA-damaging capacity	*Bacillus subtilis* M45 rec$^-$ and H17	10–5000 µg/paper disc (−S9 mix)	97	Negative −S9 mix	Kishida et al. (1980)
DNA-damaging capacity	*B. subtilis* M45 rec$^-$ and H17	100–10 000 µg/paper disc	92.5	Negative ±S9 mix	Kogiso (1986)
Gene mutation in mammalian cells	Mouse lymphoma L5178Y cells	50.3–400 µg/ml (−S9 mix) 47.5–300 µg/ml (+S9 mix)	91.4	Negative ±S9 mix	Richold et al. (1982a)
Gene mutation in mammalian cells	Chinese hamster lung cells (V79)	50–500 µg/ml (±S9 mix)	92.4	Negative ±S9 mix	Kogiso (1988)
DNA repair in mammalian cells (autoradiographic method)	HeLa S3 epithelioid cells (human cervical carcinoma)	200–3200 µg/ml (±S9 mix)	91.4	Negative ±S9 mix	Richold et al. (1982b)
Chromosomal aberration	Chinese hamster ovary cells (CHO)	50–500 µg/ml (−S9 mix) 500–5000 µg/ml (+S9 mix)	92.5	Negative ±S9 mix	Mosesso (1984)
Chromosomal aberration	Chinese hamster ovary cells (CHO-K1)	10–30 µg/ml (−S9 mix) 250–1000 µg/ml (+S9 mix)	92.0	Negative ±S9 mix	Kogiso (1989)
Chromosomal aberration	Chinese hamster ovary cells (CHO-K1)	3×10^{-6} to 10^{-4} mol/l (±S9 mix)	92.5	Negative ±S9 mix	Hara (1990)
Sister chromatid exchange	Chinese hamster ovary cells (CHO-K1)	3×10^{-6} to 10^{-4} mol/l (±S9 mix)	92.5	Negative ±S9 mix	Hara & Suzuki (1984a)

Type of study	Organism/cell line	Dose range tested	Purity (%)	Result	Reference
In vivo					
Host-mediated assay	*S. cerevisiae* JD1 – Male Carworth Farm (CF) No. 1 mice	10 and 20 mg/kg bw (orally)	97.0	Negative	Brooks (1976)
Chromosomal aberration in bone marrow	Chinese hamsters (males and females)	10 and 20 mg/kg bw (orally) for 2 consecutive days	97.0	Negative	Dean (1975)
Mouse micronucleus	Male ICR mice	50, 100 and 200 mg/kg bw (intraperitoneally)	92.5	Negative	Hara & Suzuki (1984b); Suzuki (1984)

DNA, deoxyribonucleic acid; S9, 9000 × *g* supernatant fraction of rodent liver homogenate

In addition to the above, two papers investigating possible genotoxicity of fenpropathrin were found in the public domain.

In a paper by Ryu et al. (1996), the genotoxicity of fenpropathrin was examined by means of the in vitro bacterial gene mutation and chromosomal aberration assays in mammalian cell systems and the in vivo micronucleus assay using mouse bone marrow cells. No mutagenic potential was observed, either in vitro or in vivo, but it should be noted that the chemical purity of the tested fenpropathrin was not given.

In vitro micronucleus induction by fenpropathrin (purity 99.6%) using whole blood and isolated human lymphocytes was investigated in a paper by Surrallés et al. (1995), using the cytokinesis block method with cytochalasin B (6 µg/ml). Fenpropathrin slightly increased the number of micronuclei and micronucleated cells in whole blood lymphocyte cultures from two out of three donors. Based on this result, the authors concluded that with certain reservations with respect to the purity and isomer composition of the test compound, the existing information appears to support the idea that fenpropathrin has a weak genotoxic activity in vitro. However, all of the other genotoxicity studies described above, including the in vivo micronucleus assay, were negative. The weight of evidence indicates that the in vitro effects reported in this paper have no corroboration in vivo.

2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

Rats

In a three-generation reproductive toxicity study, fenpropathrin (purity 97%) was fed to rats (COBS; 30 of each sex per group) at a dietary concentration of 0, 5, 25 or 250 ppm (equivalent to 0, 0.3, 1.7 and 16.7 mg/kg bw per day, respectively). Observations were made on health, behaviour, body weight, feed consumption and reproductive performance of each animal. Each parent animal, sacrificed at weaning of the relevant second litter, was subjected to a complete necropsy; a proportion of F_{3B} pups were also subjected to postmortem examination. Ten male and 10 female F_2 parent animals and 10 male and 10 female F_{3B} pups from the control and high-dose (250 ppm) groups were examined microscopically.

No treatment-related effects on general health, mortality, body weight gain, feed consumption or pregnancy rates were observed. Statistically significant increases in pup weights were seen in the F_{1A} generation at 250 ppm on days 7, 14 and 21. Analysis of individual male and female pup weights at weaning revealed statistically significant increases in all treated groups compared with controls. No statistically significant changes in litter weight or mean pup weight calculated from total litter weight were seen in the F_{1B} generation, although statistically significant increases in female pup weight at day 21 were observed in all treated groups. With the exception of a small decrease in male pup weight

at weaning seen in all treated groups, no litter or pup weight effects were seen in the F_2 generation that could be related to treatment. Mean pup weights in the F_{3A} generation were reduced at 250 ppm on days 14 and 21 (Table 7). This reduction was also seen in male pup weights at 25 and 250 ppm and in female pup weights at all treatment levels. No statistically significant changes in litter weight or pup weight at day 21 were seen in the F_{3B} generation; therefore, this reduction in pup weights at 250 ppm was not considered adverse (Table 8). No macroscopic or microscopic lesions were attributable to treatment. A very small number of cases of locomotor incoordination occurred in pups fed 5 and 250 ppm. Most pups in the 5 ppm group showing these signs were from one litter in the F_{1B} generation. No similar lesions were observed in the second litter of that pair or in the following generation, and no macroscopic lesions were identified in these animals. Histopathological examination (F_2 parents, F_{3B} pups) did not reveal treatment-related tissue lesions.

The NOAELs for parental toxicity and reproductive toxicity were 250 ppm (equivalent to 16.7 mg/kg bw per day), the highest dose tested. The NOAEL for offspring toxicity was 25 ppm (equivalent to 1.7 mg/kg bw per day), based on decreased pup weights in the F_{3A} generation at 250 ppm (equivalent to 16.7 mg/kg bw per day) (Fleming, 1979; Hend, Gellatly & Fleming, 1979; Else & Rushton, 1981).

In a second three-generation reproduction study, fenpropathrin (purity 92.5%) was administered to groups of 28 male and 28 female Sprague-Dawley rats (CrL: COBS CD(SD)BR) in the diet at 0, 40, 120 or 360 ppm. Mean achieved concentrations for the F_0 generation were 0, 2.6, 7.8 and 23.3 mg/kg bw per day for males and 0, 3.1, 9.1 and 27.7 mg/kg bw per day for females, respectively. Animals of the last generation (F_2) were reared to maturity. At all matings, litter values were recorded and organ weight analyses were performed on representative weanlings; organ weights were also recorded for F_0, F_{1B} and F_{2B} adults. Tissues from F_0, F_{1B} and F_{2B} adults and F_{2B} weanlings in the control and high-dose groups were submitted to histopathological examination.

There were no clear adverse effects on feed consumption, mating performance, duration of gestation or live births in the parental animals. There were no clinical signs reported for males, and no males receiving 360 ppm died. At the high dose, a total of 18 females died (2 F_0, 1 F_{1A}, 13 F_{1B} and 2 F_{2B}). Deaths of females (10) generally occurred during lactation in the F_{1B} dams. At 360 ppm, most females that littered showed body tremors associated with muscle twitches and increased sensitivity. These signs were generally observed during the lactation period. At 120 ppm, 2 of 24 F_{1B} females died during lactation, and 1 F_{1B} female showed the clinical signs observed for the high-dose group during lactation week 2; these deaths were considered related to treatment. A total of three females treated at 40 ppm died; one F_{1B} dam was killed on day 25 of gestation after showing signs of dystocia, one F_0 dam died during gestation subsequent to the second pairing (two large misshaped fetuses were found in the uterus) and one F_{1A} female died when 9 weeks old. In the absence of any clinical signs, these deaths were considered unrelated to treatment. Mean body weight gains of males in the F_{1A}, F_{1B} and F_{2B} generations were reduced at 360 ppm. F_{1B} females at 360 and 120 ppm also showed some indication of retarded weight gain. There were no clear effects on absolute body weight or body weight gain during gestation or lactation for any generation. No treatment-related macroscopic or microscopic abnormalities were observed at terminal sacrifice. Organ weight data revealed statistically significantly increased absolute and relative liver weights in F_0 females at 360 ppm.

Three F_{2B} pups at 120 ppm showed body tremors prior to weaning, and two of these pups died subsequently. A slight reduction in litter size was observed at 360 ppm in F_0 animals (second mating) and in both matings of F_{1B} animals from days 4 to 21. At 360 ppm, pup weights were lower in all generations.

The NOAEL for parental systemic toxicity was 40 ppm (equal to 2.6 mg/kg bw per day), based on mortality of two females, reduced body weights in F_{1B} females and occurrence of body tremors and muscle twitches in one F_{1B} female seen at 120 ppm (equal to 7.8 mg/kg bw per day). The NOAEL for reproductive toxicity was 360 ppm (equal to 23.3 mg/kg bw per day), the highest dose tested. The NOAEL for offspring toxicity was 40 ppm (equal to 2.6 mg/kg bw per day), based on

body tremors seen in three F_{2B} pups at 120 ppm (equal to 7.8 mg/kg bw per day) (Cozens et al., 1986).

Table 7. Litter and pup weights in a three-generation study with fenpropathrin

Generation	Litter	Dietary concentration (ppm)	Day 1		Day 4		Day 7		Day 14		Day 21	
			Mean litter weight (g)	Mean pup weight (g)	Mean litter weight (g)	Mean pup weight (g)	Mean litter weight (g)	Mean pup weight (g)	Mean litter weight (g)	Mean pup weight (g)	Mean litter weight (g)	Mean pup weight (g)
F_1	A	0	92.9	7.2	140.2	10.9	205.3	16.1	370.6	29.8	625.2	50.3
		5	101.2	7.5*	150.9	11.5*	215.2	16.6	390.6	30.9	659.5	52.3
		25	92.4	7.3	135.8	11.1	192.7	16.6	353.0	31.6	596.0	53.3
		250	91.3	7.7*	138.5	11.8**	198.9	17.0*	359.0	31.9*	620.8	55.0**
	B	0	88.5	7.5	133.2	11.6	197.5	17.4	367.1	32.6	609.9	54.3
		5	96.2	7.6	147.0	11.8	218.6	17.9	402.0	33.2	667.3	55.6
		25	93.9	7.7	143.9	12.0	212.8	18.2	385.1	33.2	636.0	54.8
		250	83.2	7.6	127.4	12.0	189.8	18.1	354.2	34.3	599.2	57.4
F_2	A	0	73.3	7.0	113.0	11.0	169.1	16.9	325.9	32.8	521.0	52.1
		5	68.3	6.7	100.6	10.6	150.9	16.3	293.8	32.5	464.4	51.5
		25	65.3	6.8	98.9	10.6	144.5	15.9	272.1	30.9	436.9	50.0
		250	71.5	7.0	106.8	10.8	161.3	16.2	296.4	31.6	483.4	51.7
	B	0	72.6	7.4	114.1	11.8	168.3	17.7	320.3	33.8	519.9	55.4
		5	55.4	7.9	86.1	12.3	129.6	18.8	241.3	35.5	364.3	53.5
		25	68.1	7.8	124.6	12.3	156.2	18.5	291.7	35.2	464.3	55.2
		250	73.3	7.7	129.6	12.2	165.1	18.2	305.6	34.4	489.9	55.2
F_3	A	0	67.4	7.3	101.7	11.3	148.7	16.8	283.4	34.1	491.5	58.4
		5	72.3	6.9	106.6	11.1	157.2	17.1	289.9	33.4	501.2	57.1
		25	72.3	6.7	107.4	10.5	152.1	15.6	274.7	31.4	482.2	55.1
		250	73.1	7.1	104.5	10.4	145.1	14.8	266.6	29.8*	456.6	51.1**
	B	0	72.2	7.5	114.0	12.2	169.5	18.4	232.9	35.6	539.3	58.7
		5	52.8	7.5	85.6	12.0	128.9	18.9	242.6	36.3	394.6	59.6
		25	69.6	7.8	10.38	12.3	149.1	18.1	270.3	33.7	454.0	55.9
		250	70.2	7.6	106.0	12.0	152.8	17.9	280.7	33.3	469.2	55.4

From Hend, Gellatly & Fleming (1979)
* $P < 0.05$; ** $P < 0.01$ (Wilcoxon’s test)

Table 8. Mean litter weight data for rats aged 21 days fed fenpropathrin during a three-generation reproductive toxicity study (from individual body weights measured at weaning)

Generation	Litter	Dietary concentration (ppm)	Males			Females		
			No. of observations	Mean pup weight (g)	% of control	No. of observations	Mean pup weight (g)	% of control
F_1	A	0	204	49.9		181	47.8	
		5	198	52.9**		186	50.4**	
		25	165	51.8**		186	50.5**	
		250	188	55.2**		156	53.3**	
	B	0	171	55.3		172	51.6	
		5	190	55.9		174	54.3	
		25	174	55.4		181	52.5	
		250	167	56.0		149	53.9**	
F_2	A	0	159	52.9		139	48.7	
		5	140	50.7*		138	50.0	
		25	126	49.9*		126	47.5	
		250	137	51.1*		135	48.3	
	B	0	135	54.9		137	52.1	
		5	107	53.3		106	50.7	
		25	114	55.1		129	52.2	
		250	125	55.0		139	51.0	
F_3	A	0	87	57.1		112	56.6	
		5	84	55.8		99	53.7*	
		25	90	54.3*		90	52.8**	
		250	109	51.1**		89	50.8**	
	B	0	104	56.3		116	56.1	
		5	60	59.8		64	54.8	
		25	85	56.5		77	55.3	
		250	81	57.3		107	53.8	

From Hend, Gellatly & Fleming (1979)
* $P < 0.05$; ** $P < 0.01$

(b) Developmental toxicity

Mice

A teratogenicity study in mice was reported, using "technical grade fenpropathrin" (purity not indicated), sourced from Rallis Agricultural Research Centre, India. Groups of 10 female Swiss albino mice were mated and then treated with fenpropathrin in corn oil at 0, 0.29 or 0.59 mg/kg bw per day during days 5–14 or 14–17 of gestation. A laparotomy was performed on day 18 of pregnancy. The number of live, dead and resorbed fetuses was recorded. Live fetuses and placental discs were removed and weighed. Fetal sex and any external abnormalities were recorded. Fetuses were then fixed and processed separately for skeletal (alizarin red; Dawson, 1926) and visceral (Wilson, 1965) examination.

Maternal toxicity was evidenced by reduced weight gain during gestation at both doses. In addition, one dam treated at the highest dose on days 14–17 of gestation died. Mean fetal weight was

decreased compared with controls. Fenpropathrin caused a dosed-related increase in the percentage of resorbed fetuses. Examination of the fetuses for external anomalies revealed the occurrence of open or slit eye in a significant number of fetuses. Other signs of teratogenicity (e.g. enlarged ventricles, microphthalmia, enlarged renal pelvis, reduced ossification of skull bones, sternebrae and extremities, presence of 13th rudimentary or absent rib) were also reported, but a number of observations from the tabular data presented failed to reveal any dose–response relationship (Dhar et al., 2004). The study does not meet Organisation for Economic Co-operation and Development guidelines for numbers of animals tested, and there is no claim of compliance with GLP. The source of the fenpropathrin is unclear.

Rats

In a developmental toxicity study, approximately 30 mated female Fischer 344 rats were given fenpropathrin (purity 96.2%; doses were corrected for purity) suspended in corn oil by oral gavage at a dose of 0, 0.4, 2 or 10 mg/kg bw per day from day 6 to day 15 of gestation. All dams were sacrificed on day 19 of gestation, and the uterine contents were examined.

One mid-dose and nine high-dose females were found dead during the study. Clinical signs noted at a somewhat greater incidence in the treated animals included soft faeces, red or lacrimating eyes, alopecia or hunched appearance. Post-dose tremors occurred on a few occasions in some high-dose females. Body weight gain was reduced in high-dose females during the treatment period and increased slightly afterwards, resulting in an overall change comparable with the control group. Feed consumption was also reduced at the highest dose level. Various gross lesions were found in animals that died during the study. The findings included discolorations of organs, but no specific target organ was identified. The pregnancy rate was lower in the high-dose group compared with the other dose groups, but other reproduction parameters (e.g. number of corpora lutea, resorptions, fetal viability) were not impaired by treatment. No visceral or skeletal anomalies were noted in the pups. Visceral or skeletal variants did not show dose-related increases. No teratogenic effects were observed.

The NOAEL for maternal toxicity was 2 mg/kg bw per day, based on decreased body weight gain and feed consumption during the dosing period and clinical signs at 10 mg/kg bw per day. The NOAEL for embryotoxicity and teratogenicity was 10 mg/kg bw per day, the highest dose tested (Pence et al., 1980c; Cox, 1986, 1987).

In a second prenatal developmental toxicity study, groups of 30 mated female Fischer 344 rats were given fenpropathrin (purity 91.9%; doses were corrected for purity) suspended in corn oil by oral gavage at a dose of 0, 0.4, 1.5, 2, 3, 6 or 10 mg/kg bw per day from day 6 to day 15 of gestation. All dams were sacrificed on day 20 of gestation, and the uterine contents were examined.

In the high-dose group, six dams died between gestation days (GDs) 7 and 13, and one was sacrificed moribund on day 8 because of convulsions and prostration. Neither death nor moribundity was found in the other groups. Clinical signs seen in the majority of high-dose dams included ataxia, sensitivity to external stimuli, spastic jumping and tremors. These signs were most severe 2 hours after dosing and during the first days of dosing, although sensitivity to external stimuli persisted throughout the dosing period. At 6 and 10 mg/kg bw per day, mean maternal body weight gains and feed consumption were lower during the dosing period (Table 9). Body weight gain was significantly decreased in the 10 and 6 mg/kg bw per day groups between GDs 6 and 8, 6 and 11, 6 and 15 and 6 and 16. Decreases ranged from 29% to more than 97% for the high-dose group and from 14% to 80% at 6 mg/kg bw per day. Over the course of the study (GDs 0–20 or 6–20), however, body weights, gravid uterine weights, corrected body weights and feed consumption were similar in all groups. The corrected body weight (terminal weight minus gravid uterine weight) for the high-dose dams was significantly less (4%) than the control value, and the net weight change (corrected weight minus day 0 body weight) was also significantly less (40%) than the control value. For the 6 mg/kg bw per day group, there was a non-significant 16% reduction in the net weight change. Feed consumption was also significantly decreased (18% or 11%) in the 10 or 6 mg/kg bw per day groups, respectively, between GDs 6 and 8. Between GDs 8 and 11, there was a significant reduction (7%) in feed

consumption for the 10 mg/kg bw per day group. Throughout the remainder of the study, feed consumption was unaffected by treatment.

Table 9. Mean maternal body weights during gestation

Interval		Dose (mg/kg bw per day) (no. of dams)						
		0 (*n* = 30)	0.4 (*n* = 28)	1.5 (*n* = 28)	2 (*n* = 29)	3 (*n* = 28)	6 (*n* = 28)	10 (*n* = 27–21)
Pretreatment	Day 0	170	171	173	173	172	174	172
Treatment	Day 6	180	181	184	182	180	183	181
	Day 8	184	184	187	186	184	184	180
	Day 11	192	193	195	195	191	192	187
	Day 15	203	204	207	206	202	203	197
Post-treatment	Day 16	208	210	212	211	207	207	201
	Day 20	236	240	242	241	237	239	233
Corrected body weights[a]		195	195	200	198	195	195	188* (−4%)
Net weight change[b]		25	24	26	26	23	21 (−16%)	15** (−40%)

From Morseth (1990)
* $P < 0.05$; ** $P < 0.01$
[a] Corrected weight = terminal body weight – gravid uterine weight.
[b] Net weight change from day 0 = corrected weight – day 0 body weight.

The pregnancy rate of high-dose females was slightly lower (90%) than that of the other treatment groups (93–97%). Treatment had no influence on reproduction parameters, such as resorptions, fetal viability or fetal body weight. No visceral or skeletal anomalies were observed that were considered treatment related. Skeletal variations and differences in ossifications were noted in all groups without a clear dose–response relationship, and statistical significances were not achieved.

The NOAEL for maternal toxicity was 3 mg/kg bw per day, based on slightly decreased body weight gain and feed consumption, mortality and clinical signs at 6 mg/kg bw per day. The NOAEL for developmental toxicity was 10 mg/kg bw per day, the highest dose tested (Morseth, 1990).

Rabbits

In a prenatal developmental toxicity study, groups of 20–31 mated female banded Dutch rabbits were given fenpropathrin in corn oil (purity 97%) by means of two gelatine capsules at a dose of 0, 0 (100 µl/kg bw corn oil control), 1.5, 3 or 6 mg/kg bw per day from day 6 to day 18 (inclusive) of gestation. All does were sacrificed on day 28 of gestation, and the uterine contents were examined for viability and teratological changes.

No clinical signs of intoxication were observed. There were several mortalities among animals treated at 3 and 6 mg/kg bw per day, but the causes of death were unrelated to treatment (dosing injuries, pneumonia and pleuritis associated with *Pasteurella multocida* infection, haemorrhage in the pleural cavity). One rabbit in the corn oil group (day 28), one rabbit in the 1.5 mg/kg bw per day group (day 27) and two rabbits in the 3 mg/kg bw per day group (days 27 and 28) aborted, with one of those at 3 mg/kg bw per day dying shortly afterwards. Treatment had no effect on body weight of the dams. No adverse effects were observed with respect to preimplantation loss,

number of resorptions, fetal deaths, litter size or pup weight. Increased incidences of minor skeletal and visceral abnormalities (e.g. enlarged fontanelles, interparietal bones, fused sternebrae, extra centres of ossification) were noted at 3 mg/kg bw per day, but not at the highest dose. Therefore, these changes were not considered treatment related.

The NOAELs for maternal toxicity, embryotoxicity and teratogenicity were 6 mg/kg bw per day, the highest dose tested (Van der Pouw et al., 1975; Dix, 1985).

In a second prenatal developmental toxicity study, groups of 17–19 mated female New Zealand White rabbits received fenpropathrin (purity 92.5%) in corn oil by oral gavage at a dose of 0, 4, 12 or 36 mg/kg bw per day daily from day 7 to day 19 of gestation. All does were sacrificed on day 29 of gestation, litter parameters determined and fetuses subsequently examined for visceral and skeletal anomalies.

A dose-related increase in the incidence of grooming was observed in all dose groups, which was nonspecific and therefore not considered as adverse. At 12 and 36 mg/kg bw per day, flicking of forepaws was also observed, with a few animals at 36 mg/kg bw per day showing shaky movements and tremor. Among the several animals dying during the study, one and two dams at 12 and 36 mg/kg bw per day, respectively, aborted, and one from each group was sacrificed because of poor conditions. None of the mortalities occurring in the study was considered to be treatment related. Slight reductions in body weight gain and reduced feed consumption were noted at 36 mg/kg bw per day. There was no effect of treatment on litter parameters or increased incidence of skeletal anomalies.

The maternal NOAEL was 4 mg/kg bw per day, based on clinical signs noted at 12 mg/kg bw per day. The developmental NOAEL was 36 mg/kg bw per day, the highest dose tested (Cozens, 1985).

2.6 *Special studies*

(a) Acute neurotoxicity

Mice

The aim of a study published by Nieradko-Iwanicka & Borzęcki (2010) was to investigate a potential hazard to memory for elderly patients undergoing a transient ischaemic event. The model used bilateral clamping of carotid arteries, a model for ischaemia, together with fenpropathrin (stated purity 99%) given intraperitoneally, testing memory, movement activity and coordination in mice. In this study, female Swiss mice with bilateral clamping of carotid arteries were injected (intraperitoneally) 24 hours after their surgery with 0.1 LD_{50} fenpropathrin (23.8 mg/kg bw). The authors concluded that "BCCA [bilateral clamping of carotid arteries] together with fenpropathrin significantly reduced latency in a passive avoidance task compared to controls. There were no significant differences among the groups with respect to the Y-maze, movement activity, or movement coordination".

Rats

In a special study, the neurotoxic effect of fenpropathrin in rats was assessed by the mean slip angle test. Groups of 10 male Sprague-Dawley rats received fenpropathrin (purity 93.8%) in corn oil once by oral gavage at a dose of 0, 10, 25, 50, 75 or 100 mg/kg bw at a volume of 5 ml/kg bw. The determination of the mean slip angle was carried out by using an originally designed apparatus. The determination of mean slip angle was performed on day 0 (just before dosing), 1, 3, 5, 7 and 9 hours after dosing and daily thereafter for 3 days.

The mean slip angle in the control animals was progressively but slightly lowered from the initial value. The mean slip angle in rats dosed at 10 and 25 mg/kg bw was comparable with that of the controls at each determination. The mean slip angle at 50 mg/kg bw and above was progressively lowered 3–5 hours after dosing. The mean slip angle of all treated groups was comparable with that of the controls 24 hours after dosing and thereafter. A total of two, five and seven rats died, respectively,

following administration of 50, 75 and 100 mg/kg bw. All deaths occurred between 5 hours and 1 day after dosing.

Slight tremors (noted as trembling of the root of the tail) were noted in rats treated at 25 mg/kg bw from 3 to 9 hours after dosing. In rats treated at 50 mg/kg bw, slight to moderate tremor (trembling limbs) appeared at 3 hours. Ataxia, limb paralysis and acoustic startle response to sound were also observed at 5–7 hours. Slight to moderate tremors appeared at 1 hour in animals treated at 75 and 100 mg/kg bw. The severe tremor, ataxia, limb paralysis and acoustic startle response were observed at 5–9 hours post-dosing at 75 mg/kg bw and at 3–7 hours at 100 mg/kg bw. These clinical signs completely disappeared in most of surviving animals within 24 hours of administration.

The NOAEL of this study was 10 mg/kg bw, based on tremors observed at 25 mg/kg bw (Hiromori et al., 1986a).

Functional testing was performed on two animals of each sex at 0, 125 or 500 ppm (equivalent to 0, 6.25 and 25 mg/kg bw per day), satellite groups of the long-term study in rats (Hend & Gellatly, 1979), using the “inclined plane test”. Preliminary results revealed an impaired performance in animals fed 500 ppm. Measurements of β-glucuronidase activity (parameter indicative of Wallerian degeneration in nerves) did not reveal a clear increase, as would be expected as a result of toxic neuropathy. Macroscopy and histopathology of sciatic and tibial nerves did not show treatment-related changes (Hend et al., 1980).

Wolansky et al. (2006) reported on a study in which motor activity effects were used as an end-point to determine a dose–response curve and relative potency after acute exposure to 11 commonly used pyrethroids (Type I: bifenthrin, *S*-bioallethrin, permethrin, resmethrin, tefluthrin; Type II: beta-cyfluthrin, lamda-cyhalothrin, cypermethrin, deltamethrin, esfenvalerate; and one mixed Type I/II: fenpropathrin). In this study, adult male Long-Evans rats were administered each test chemical as a single gavage dose in corn oil (1 ml/kg bw); each group consisted of 8–18 animals, and each experiment was divided into at least two blocks, with vehicle control animals included in each block. Motor activity was measured for 1 hour using 16 figure-eight mazes at the time of peak effects. The time of peak effects was obtained from pilot time course studies using motor activity testing or behavioural observations. The motor activity data were analysed using a non-linear exponential threshold activity model (Casey et al., 2004). Relative potencies were calculated from ED_{30}s (defined as the dose associated with a 30% decrease in motor activity) using deltamethrin as the index chemical. Fenpropathrin (purity 91.8%) was given at eight doses ranging between 0.01 and 24 mg/kg bw and tested for motor activity 2 hours after dosing. Excessive signs (i.e. prolonged [lasting more than 4 hours] clinical signs) were observed at 24 mg/kg bw, and this dose was not used in any data analysis.

All pyrethroids, including fenpropathrin, induced dose-dependent decreases in motor activity. The threshold dose is defined as an estimate of the highest no-effect level at which treated rats did not display any decreases in motor activity. The threshold dose for fenpropathrin was 3.06 mg/kg bw, with a 95% confidence interval of 1.76–4.37 mg/kg bw. The ED_{30} for fenpropathrin was 7.70 ± 0.65 mg/kg bw (Wolansky et al., 2006).

In a range-finding study for acute neurotoxicity assessments, fenpropathrin (purity 92%) in corn oil was administered orally by gavage as a single dose to three Crl:CD(SD) rats of each sex per group at a dose level of 6, 24, 36 or 54 mg/kg bw. A concurrent control group (three of each sex) received the vehicle on a comparable regimen. The dosage volume was 5 ml/kg bw for all groups. Dietary stability, homogeneity and concentrations were confirmed analytically. All animals were observed twice daily for mortality and moribundity. Individual body weights were recorded on the day of test substance administration (day 0). Clinical examinations were conducted simultaneously

with modified functional observational battery evaluations. Modified functional observational battery data were recorded for all animals at approximately 1, 2, 3, 4, 5, 6, 7 and 8 hours following dose administration. All animals found dead or euthanized in extremis received a complete necropsy. All surviving animals were euthanized and discarded following the completion of the functional observational battery evaluation for each animal.

One male in the 54 mg/kg bw group died following the 4-hour post-dosing modified functional observational battery, and one female in each of the 36 and 54 mg/kg bw groups was euthanized in extremis immediately following the 6- and 4-hour modified functional observational battery evaluations; these deaths were attributed to treatment with fenpropathrin and indicate that these dose levels were above the MTD. The predominant findings noted at the modified functional observational batteries were convulsions, tremors, Straub tail and altered gait. Time of peak effect was 3 hours post-dosing for males and females. Signs of neurotoxicity were most severe and were observed at the highest incidence at 36 and 54 mg/kg bw. Treatment-related effects on modified functional observational battery parameters at the time of peak effect in the 24 mg/kg bw group included tremors and gait alterations (walking on tiptoes or hunched appearance). In addition, one female in this group was noted with Straub tail. Slight tremors and altered gait were noted at 6 mg/kg bw. However, in a following range-finding study (Knapp, 2006b), seven animals of each sex per group were evaluated at 3 and 6 mg/kg bw, and there were no clinical signs observed. In addition, there were no test substance–related effects on locomotor activity evaluations. Therefore, it was uncertain whether these findings (slight tremors and altered gait) in the 6 mg/kg bw group in this study were treatment related. No treatment-related effects were observed macroscopically for the animals that were found dead or euthanized in extremis (Knapp, 2006a).

In a second range-finding study for acute neurotoxicity assessments, fenpropathrin (purity 92.0%) in the corn oil vehicle was administered orally by gavage as a single dose to two groups of seven Crl:CD(SD) rats of each sex per group at a dose level of 3 or 6 mg/kg bw. A concurrent control group (group 1) received the vehicle on a comparable regimen. The dosage volume was 5 ml/kg bw for all groups. The other experimental details mentioned in the above-described range-finding study (Knapp, 2006a) were followed.

No treatment-related effects on survival and modified functional observational battery or locomotor activity evaluations performed at the time of peak effect (approximately 3 hours after treatment) were noted following a 3 or 6 mg/kg bw oral gavage dose (Knapp, 2006b).

In an acute neurotoxicity study (main study), fenpropathrin (purity 92.0%) in corn oil was administered once via gavage (5 ml/kg bw) to 12 Crl:CD(SD) rats of each sex per group at a dose level of 0, 3, 6, 15 or 30 mg/kg bw. Dietary stability, homogeneity and concentrations were confirmed analytically. All animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily. Individual body weights were recorded weekly. Functional observational battery and locomotor activity data were recorded for all animals prior to the initiation of dose administration, at the time of peak effect (approximately 3 hours post-dosing) on study day 0 and on study days 7 and 14. All animals were euthanized on study day 15 and perfused in situ with a buffered 4.0% paraformaldehyde/1.4% glutaraldehyde solution. Brain weights (excluding olfactory bulbs) and brain dimensions were recorded for each of these animals. In addition, a neuropathological evaluation was performed on six animals of each sex in the control and 30 mg/kg bw groups. Acceptable positive control data were provided.

No compound-related effects on mortality, clinical signs of toxicity, body weight, body weight gain, motor activity, brain weight and morphology, gross pathology or neuropathology (30 mg/kg bw group) were observed in either sex.

At 30 mg/kg bw, the following functional observational battery effects (no. affected/12 versus 0/12 controls) were noted at 3 hours post-dosing (time of peak effect): 1) slight tremors in 10 animals of each sex during the open-field observations; 2) clonic convulsions (whole-body tremors) in one rat

of each sex during the open-field observations; and 3) slight tremors in two males and one female during the home cage observations. These behavioural changes were apparently reversible, as all animals appeared normal by day 7. Findings at 15 mg/kg bw were limited to slight tremors in two females at 3 hours post-dosing during the open-field observations. In the absence of other neurotoxic effects in this group and the absence of any findings in the 15 mg/kg bw males, this finding is considered equivocal for neurotoxicity. Handling, sensorimotor, neuromuscular and physiological observations were not affected on study day 0. No treatment-related effects were apparent when functional observational battery evaluations were performed on study days 7 and 14. A significant decrease in overall total motor activity counts was obtained in males treated at 30 mg/kg bw (147.67; 9.7% reduction in total activity) during the time of peak effect compared with the mean control group value (163.60). However, owing to the magnitude of the difference, this decrease was not considered related to treatment. No effects were apparent in mean ambulatory and total motor activity counts on study days 7 and 14. No remarkable shifts in the pattern of habituation occurred in any of the treated groups when the animals were evaluated on study days 0, 7 and 14.

The NOAEL for neurotoxicity was 15 mg/kg bw, based on slight tremors and clonic convulsions (whole-body tremors) in both sexes at the time of peak effect at 30 mg/kg bw (Knapp, 2007a). However, the registrant established a no-observed-effect level (NOEL) for neurotoxicity of 15 mg/kg bw for males and 6 mg/kg bw for females, probably because the registrant considered tremors (two rats) seen at the 15 mg/kg bw as an adverse effect.

In a study published by Weiner et al. (2009), 12 commercial pyrethroid insecticides (technical-grade active ingredients) were evaluated for acute neurobehavioural manifestations of toxicity under conditions suited to assist with determining whether they act by a common mechanism of toxicity. Young adult male Sprague-Dawley rats (10 per dose group) were administered a single dose of pyrethroid by oral gavage, in corn oil, at a volume of 5 ml/kg bw. Each was tested at a range of two or three dose levels, including a minimally toxic dose, to establish the more sensitive manifestations of toxicity, and a more toxic dose, to establish a more complete spectrum of neurobehavioural manifestations. Animals were evaluated using a functional observational battery that was designed to characterize and distinguish effects classically associated with tremor or choreoathetosis with salivation syndromes of intoxication. The functional observational battery was performed at the time of peak effect (2, 4 or 8 hours post-dosing) by observers who were blinded to dose group assignment. The dose levels for fenpropathrin were 15 and 30 mg/kg bw.

The results indicate that some pyrethroids clearly exhibit the historic classification symptoms of the tremor or choreoathetosis with salivation syndromes, whereas others do so less obviously. Use of the statistical technique of principal component analysis further helped interpret the study findings. Based on a review of the functional observational battery data, analysed by principal component analysis, and other published data, two common mechanism groups were proposed. Group 1 would include pyrethrins, bifenthrin, resmethrin, permethrin, *S*-bioallethrin and tefluthrin. Group 2 would include cypermethrin, deltamethrin, esfenvalerate, beta-cyfluthrin and lamda-cyhalothrin. Fenpropathrin exhibited features of both groups (Weiner et al., 2009). Assessments performed in this study did not include motor activity, so the relative effectiveness of motor activity compared with other functional observational battery assessments was not determined.

(b) Subchronic neurotoxicity

Hens

The neurotoxic potential of fenpropathrin was investigated in adult laying hens in a study that predated GLP. Six adult domesticated laying hens were given five successive daily doses of fenpropathrin (purity 96%) dissolved in dimethyl sulfoxide at 1000 mg/kg bw. The dosing regimen was repeated after a 3-week treatment-free period, and the birds were sacrificed a further 3 weeks later. A positive control group was pretreated with an intramuscular dose of pralidoxime chloride and atropine sulfate and dosed with 0.5 ml/kg bw of tri-*ortho*-tolyl phosphate (TOTP). A third group,

which was not dosed, acted as the negative control. All birds were observed daily and tested at intervals for their ability to land properly when forced to fly.

All birds receiving the positive control dose of TOTP developed signs of neurological damage and lesions in the nervous system. None of the birds dosed with fenpropathrin showed any signs of intoxication. Histological examination of their nervous system showed no lesions (Milner & Butterworth, 1977).

Rats

In a dietary 28-day range-finding study for a subsequent subchronic neurotoxicity study, groups of six Crl:CD(SD) rats of each sex per dose received fenpropathrin (purity 92%) continuously in their diet for 28 consecutive days at a concentration of 0, 60, 300 or 600 ppm (equal to 0, 5, 26 and 53 mg/kg bw per day for males and 0, 6, 31 and 64 mg/kg bw per day for females, respectively). Stability, homogeneity and concentrations in the diets were confirmed analytically. All animals were observed twice daily for mortality and moribundity. Detailed physical examinations, body weights and feed consumption were recorded weekly. Functional observational battery and locomotor activity data were recorded for all animals during study week 3. Gross necropsies were conducted on all animals, and selected organs were weighed.

No treatment-related effects on mortality, brain weight or gross pathology were noted in either sex. In the 300 ppm males, decreases were noted in cumulative body weight gain from week 0 to week 3 (16%) and overall (20%, weeks 0–4) and in feed efficiency (32%) during weeks 3–4. At 600 ppm, clinical signs of toxicity observed included tremors, head and/or body twitches and/or hypersensitivity to sound in both sexes; and popcorn seizures (two females on day 5) and red and/or yellow material on the ventral abdominal and/or urogenital areas in the females. Body weights were decreased throughout the study by 9–12% in the males and by 8–12% in the females. Body weight gains were decreased by 38% in the males and by 48% in the females during the 1st week of treatment. Cumulative body weight gains were decreased throughout the study by 26–38% in the males and by 29–48% in the females, which resulted in overall (weeks 0–4) gains being decreased by 26–29% in both sexes. During the 1st week of treatment, decreases in feed consumption (12–19%) and feed efficiency (29–36%) were also noted in both sexes. The following functional observational battery effects (no. affected/6 versus 0/6 controls) were noted: 1) during the home cage observations, clonic convulsions, whole-body convulsions and markedly coarse tremors were observed in one female; 2) during handling, soiled fur was noted in two females; 3) during open-field observations, five females displayed abnormal gait (walking on tiptoes or dragging hindlimbs), and one female was ataxic; four females displayed slight to moderately coarse tremors, and two females displayed slight to considerable mobility impairment; and 4) during sensory observations, two females displayed an increased startle response, and one female displayed slightly uncoordinated air-righting reflex, whereas two other females landed on their backs. Overall session total and ambulatory motor activities were decreased in the females. Habituation to the test environment was generally noted in these animals.

The NOAEL was 60 ppm (equal to 5 mg/kg bw per day), based on decreased body weight gain and feed efficiency in males at 300 ppm (equal to 26 mg/kg bw per day) (Knapp, 2007b).

In another subchronic neurotoxicity study, fenpropathrin (purity 92%) was administered orally via the diet for 90 consecutive days to groups of 12 Crl:CD(SD) rats of each sex per dose at a dose level of 0, 60, 190 or 570 ppm (equal to 0, 4, 13 and 38 mg/kg bw per day for males and 0, 5, 15 and 50 mg/kg bw per day for females, respectively). Stability, homogeneity and concentrations in the diet were confirmed analytically. All animals were euthanized following 13 weeks of treatment. All animals were observed twice daily for mortality and moribundity. Detailed physical examinations were performed weekly. Individual body weights and feed consumption were recorded weekly. Functional observational battery and locomotor activity data were recorded for all animals prior to the initiation of treatment and during study weeks 3, 7 and 12. Ophthalmic examinations were performed during study weeks −2 and 11. All animals were euthanized and perfused in situ with a buffered 4.0%

paraformaldehyde/1.4% glutaraldehyde solution. Brain weights (excluding olfactory bulbs) and brain dimensions were recorded for each of these animals. In addition, a neuropathological evaluation was performed on six animals of each sex in the control and 570 ppm groups. A gross necropsy was performed on the animal found dead.

No compound-related effects were observed in ophthalmoscopic examinations, motor activity, brain weight and morphology, or gross pathology and neuropathology in either sex. At 570 ppm, one female that displayed tremors and hypersensitivity to sound during the first 6 days of test article administration was found dead on day 7. The following clinical signs of toxicity were observed (no. affected/12 versus 0/12 controls): 1) tremors (10 males and 12 females); 2) hypersensitivity to sound (12 rats of each sex); and 3) popcorn seizures (4 females). The frequency of tremors and hypersensitivity to sound were greater in the females than in the males. At 570 ppm, body weights were decreased throughout the study by 11–13% in the males and by 8–13% in the females. Feed consumption was decreased (10–19%) in males throughout the study. Mean feed efficiency (body weight gained as a percentage of feed consumed) was decreased by 27% in the males during week 0–1 and by 29–59% in the females during weeks 0–1, 1–2 and 4–5 compared with controls.

Differences in functional observational battery parameters were noted in animals of both sexes treated at 570 ppm during the study week 3, 7 and/or 12 evaluations, with the greatest incidence of findings occurring in the females treated at 570 ppm. Treatment-related functional observational battery effects were predominantly observed in the 570 ppm females and included the following (no. affected/11 females versus 0/12 controls): During the home cage observations, slight to markedly coarse tremors were noted in 3–5 females throughout the treatment period. During the handling observations, soft and flabby muscle tone was noted in three females during week 3. In the open field, slight to markedly coarse tremors were noted in 3–7 females throughout the treatment period and in 1 male at week 3; slightly to moderately impaired mobility was noted in 2–3 females during weeks 3 and 12; clonic convulsions (whole-body tremors) were observed in 3 females during weeks 3 and 7; slight to moderately impaired gait score was noted in 2–3 females throughout the treatment period, and 1 male displayed body dragging during week 3; abnormal gait (walking on tiptoes) was noted in 2 females at week 12, and hunched body was noted in 3 females at week 12; and the mean number of rears was increased by 91% in the males during week 3 and by 100% in the females during week 12. During the sensory observations, a more energetic startle response was noted in 3 females during weeks 3 and 12, and slightly uncoordinated air-righting reflex was noted in 1–3 females during weeks 3 and 12. Also during the air-righting reflex testing, 1 additional female landed on its side or back during weeks 3 and 7, respectively. During the neuromuscular observations, mean hindlimb grip strength was decreased by 27% in the females during week 3; rotarod performance was decreased by 51–68% in the females throughout treatment; and hindlimb foot splay was decreased by 34% in the females during week 3. Additional findings observed at this dose in the home cage or open field included ataxia in 2 females during week 3 and in 1 female at week 7, whole-body tremors in 1 female at weeks 3 and 12, biting the cage or self in 2 females at week 12, and popcorn seizure in 1 female each at weeks 3 and 7. Effects noted at 190 ppm were limited to abnormal gait (2 females each displayed walking on tiptoes and hunched body) at week 12. There were no treatment-related clinical findings noted in animals of either sex treated at 60 ppm or in males treated at 190 ppm; body weights and feed consumption at these dose levels were unaffected by treatment. There were no treatment-related effects on handling observations during the functional observational battery evaluations. Locomotor activity was unaffected by test article administration at all dose levels.

In summary, neurotoxicity of fenpropathrin administered via diet was observed at dose levels of 190 ppm in females (equal to 15 mg/kg bw per day) and 570 ppm in males and females (equal to 38 and 50 mg/kg bw per day, respectively). Neurotoxic effects were evidenced by walking on tiptoes and hunched body (females at 190 ppm); abnormal gait and tremors (570 ppm, both sexes); convulsions, excessive biting, impaired mobility, vertical jumping, increased rearing, uncoordinated righting reflex, excessive startle response and lower hindlimb grip strength, foot splay and rotarod performance (570 ppm, females).

The NOAEL in females was 60 ppm (equal to 5 mg/kg bw per day), based on abnormal gait (two females each displayed walking on tiptoes and hunched body) at 190 ppm (equal to 15 mg/kg bw per day) at week 12 (Knapp, 2007c).

(c) Developmental neurotoxicity

Rats

A preliminary range-finding study was conducted to determine the dietary concentrations of the test substance appropriate for use in the definitive developmental neurotoxicity study and to provide data regarding the level of exposure of the offspring at critical periods of development (GD 20 and lactation days [LDs] 4, 10 and 16) following maternal dietary exposure during gestation and lactation. In a dose range–finding developmental neurotoxicity study, fenpropathrin (purity 92.0%) was administered in the diet to two groups of presumed pregnant Sprague-Dawley (Crl:CD[SD]) rats per dose at a dose of 0, 50, 180 or 360 ppm (equal to 0, 4, 13 and 27 mg/kg bw per day during gestation and 0, 7, 25 and 44 mg/kg bw per day during lactation, respectively). In Subset I, 5 females per dose group were dosed continuously from GD 6 to GD 20; in Subset II, 10 females per dose group were dosed continuously from GD 6 through LD 21. Subset I females were killed on GD 20, and their pregnancy status was determined by caesarean section. Subset II females were allowed to litter normally and rear their offspring to LD 21. The pups were not directly dosed. On postnatal day (PND) 4, litters were randomly standardized to eight pups per litter (four of each sex where possible). In addition to the normal systemic measurements, concentrations of the test substance in the plasma were measured in the Subset I females and their fetuses on GD 20, and concentrations of the test substance in the milk and plasma were measured in the Subset II females (five females per dose group for milk and plasma, respectively) and their pups on LD 4 (culled pups pooled by litter), LD 10 (two pups of each sex per litter, pooled by sex within litter) and LD 16 (one pup of each sex per litter). Motor activity assessments were performed on one Subset II pup of each sex per litter on PND 21. All Subset II dams and surviving pups were killed and necropsied on PND 21.

There were no effects of treatment observed on mortality, pregnancy status, mean gestation duration, the process of parturition or macroscopic pathology.

At 360 ppm, two dams had total litter losses: one on LD 16 and the other on LD 20. Slight tremors were observed in 14 of 15 dams during gestation; 5 of these animals also had piloerection on 1–3 occasions during GDs 17–20. In the Subset II females, slight tremors were noted in all animals generally beginning on LD 0; tremors were noted on most days during lactation until termination. The severity generally progressed from slight to moderate to severe, with dams used for milk collection affected to a greater extent than those used for plasma collection. This was attributed to the greater compound consumption later in lactation due to the increased maternal demand on the milk collection females; the litter sizes of these females were approximately twice the sizes of the litters of the plasma collection females, whose offspring were being killed for plasma collection on LDs 10 and 16.

Additionally at this dose, body weights were decreased ($P \leq 0.05$) by 5–8% during GDs 9–20, resulting in decreased body weight gains during GDs 6–20 (22%; $P \leq 0.01$). Body weights were generally decreased ($P \leq 0.05$) throughout lactation in both plasma and milk collection females (9–18%; not significant on LD 21). Overall (LDs 1–21) lactational body weight gains were decreased (not significantly) by 59%, and overall absolute lactational feed consumption (g/rat per day) and relative lactational feed consumption (g/kg bw per day) were decreased ($P \leq 0.01$) by 32% and 23%, respectively, in the milk collection females.

There were no effects of treatment on the number of pups born, sex ratio at birth, live litter size on PND 0, live birth index or macroscopic pathology.

At 360 ppm, the weaning index was decreased (82.5% versus 100%; not significant) for the milk collection pups. Seven blood collection and nine milk collection pups were found dead; eight blood collection pups (litter no. 27772) had cool bodies on PND 7; and 15 milk collection pups (eight pups in litter no. 27834 and seven pups in litter no. 27788) were noted with cool bodies during PNDs 6–21.

Additionally at this dose, pup body weights were decreased from PND 4 (pre-cull) to PND 21 in both the blood and milk collection pups (20–58%; $P \le 0.05$, except not significant for the blood collection males on PNDs 17 and 21), resulting in decreased body weight gains for all intervals (47–92%; $P \le 0.05$, except not significant for the blood collection males and 0.1 g body weight loss for the blood collection females on PNDs 17–21). During motor activity testing, neither the males nor females showed habituation on PND 21. This was considered to be indicative of a developmental delay, which was corroborated by the decreases in body weights and body weight gains, and not an indication of developmental neurotoxicity.

Concentrations of the test compound were lower in fetal plasma than in maternal plasma at all dietary concentrations on GD 20. The concentration of fenpropathrin in maternal plasma samples collected on GD 20 increased with increasing dietary concentration. Fenpropathrin was also measurable in the plasma of fetuses in the 180 and 360 ppm groups, but was not measurable in the fetuses in the 50 ppm group. Maternal and fetal plasma fenpropathrin concentration data are summarized in Table 10.

Table 10. Plasma fenpropathrin concentrations on PND 20

PND sample	Mean levels (ppm) of fenpropathrin ± SD in plasma; (range)/*N*			
	0 ppm	50 ppm	180 ppm	360 ppm
Day 20 dams	< LOQ[a]/5	0.021 ± 0.006 (0.011–0.027)/5	0.073 ± 0.035 (0.043–0.126)/5	0.154 ± 0.068 (0.092–0.268)/5
Day 20 fetuses (litter means)	< LOQ/5	< LOQ/5	0.038 ± 0.015 (0.018–0.056)/5	0.056 ± 0.020 (0.032–0.077)/5

From Stump (2007)
LOQ, limit of quantification; SD, standard deviation
[a] Calculated concentration was less than 0.01 ppm.

Concentrations of fenpropathrin in maternal plasma samples increased with increasing dietary concentration on LDs 4, 10 and 16. Mean concentrations were relatively similar for each dose on all days of testing, although there was some variability in individual concentrations. Fenpropathrin was also measurable in the plasma of male and female pups on all days of testing. Concentrations in these pups increased in an exposure-related manner on PNDs 4 and 10, but not on PND 16. Across groups, mean plasma concentrations in the pups were 44% and 64% of maternal plasma concentrations on PNDs 4 and 10, whereas on PND 16, plasma concentrations in the pups were 70–85% of the maternal values at 50 and 180 ppm and approximately 30% of the maternal values at 360 ppm. The lower pup to maternal plasma fenpropathrin concentration ratios in the 360 ppm groups correspond to the severe developmental delays in this group, which resulted in the pups initiating self-feeding later in the postnatal period. Maternal and fetal plasma fenpropathrin concentration data during lactation are summarized in Table 11.

Concentrations of fenpropathrin in milk increased with dietary concentration and also generally increased as lactation progressed, although the values on LDs 10 and 16 were not remarkably different, which was consistent with the calculated test article consumption values for all groups for these time frames. Maternal milk fenpropathrin concentration data are summarized in Table 12.

The levels of fenpropathrin in plasma and milk were approximately 2- to 3.4-fold higher in dams compared with pups. The NOAEL for maternal toxicity was 180 ppm (equivalent to 13/25 mg/kg bw per day during gestation/lactation, respectively), based on total litter losses, tremors, decreased gestational and lactational body weights and body weight gains, and decreased absolute and relative lactational feed consumption at 360 ppm (equivalent to 27/44 mg/kg bw per day during gestation/lactation, respectively).

Table 11. Plasma fenpropathrin concentrations during lactation

PND sample	Mean levels (ppm) of fenpropathrin ± SD in plasma; (range)/*N*			
	0 ppm	50 ppm	180 ppm	360 ppm
Day 4 dams	< LOQ[a]/5	0.0594 ± 0.017 (0.038–0.075)/5	0.265 ± 0.050 (0.215–0.325)/5	0.555 ± 0.17 (0.387–0.781)/5
Day 4 pups (pooled litter means)	< LOQ/5	0.036 ± 0.014 (0.022–0.054)/5	0.133 ± 0.053 (0.091–0.225)/5	0.269 ± 0.086 (0.161–0.394)/5
Day 10 dams	< LOQ/5	0.070 ± 0.021 (0.041–0.087)/4	0.253 ± 0.060 (0.187–0.350)/5	0.559 ± 0.130 (0.399–0.743)/5
Day 10 male pups (2/litter)	< LOQ/5	0.038 ± 0.011 (0.025–0.051)/5	0.155 ± 0.033 (0.118–0.192)/5	0.245 ± 0.160 (0.097–0.498)/5
Day 10 female pups (2/litter)	< LOQ/5	0.039 ± 0.009 (0.028–0.049)/5	0.162 ± 0.022 (0.134–0.191)/5	0.266 ± 0.0120 (0.142–0.455)/5
Day 16 dams	< LOQ/5	0.061 ± 0.014 (0.050–0.080)/4	0.242 ± 0.035 (0.181–0.267)/5	0.454 ± 0.100 (0.337–0.550)/5
Day 16 male pups (2/litter)	< LOQ/5	0.043 ± 0.015 (0.028–0.064)/5	0.198 ± 0.054 (0.127–0.256)/5	0.135 ± 0.054 (0.073–0.165)/2
Day 16 female pups (2/litter)	< LOQ/5	0.050 ± 0.016 (0.033–0.072)/5	0.206 ± 0.075 (0.118–0.313)/5	0.150 ± 0.025 (0.132–0.167)/2

From Stump (2007)
LOQ, limit of quantification; SD, standard deviation
[a] Calculated concentration was less than 0.01 ppm.

Table 12. Milk fenpropathrin concentrations during lactation

PND sample	Mean levels (ppm) of fenpropathrin ± SD in milk; (range)/*N*			
	0 ppm	50 ppm	180 ppm	360 ppm
Day 4	< LOQ[a]/5	2.55 ± 0.30 (2.17–2.85)/5	10.2 ± 7.0 (5.42–20.4)/4	22.2 ± 11.0 (5.94–34.6)/5
Day 10	< LOQ/5	3.61 ± 1.2 (2.69–5.69)/5	18.8 ± 5.6 (11.2–24.6)/4	31.9 ± 5.9 (23.5–38.6)/5
Day 16	< LOQ/5	3.74 ± 1.3 (2.65–6.00)/5	17.0 ± 5.0 (11.4–22.4)/4	30.8 ± 9.0 (20.1–44.5)/5

From Stump (2007)
LOQ, limit of quantification; SD, standard deviation
[a] Calculated concentration was less than 0.01 ppm.

The NOAEL for offspring toxicity was 180 ppm (equivalent to 13/25 mg/kg bw per day during gestation/lactation, respectively), based on decreased weaning index, pups with cool bodies, and decreased body weights and body weight gains at 360 ppm (equivalent to 27/44 mg/kg bw per day during gestation/lactation, respectively).

A NOAEL for developmental neurotoxicity could not be assigned, as insufficient parameters were examined to determine this end-point.

Based on the maternal milk and pup plasma concentrations of fenpropathrin, the dietary route of administration was confirmed to be appropriate for a developmental neurotoxicity study in rats (Stump, 2007).

In a developmental neurotoxicity study (main study), fenpropathrin (purity 92.0%) was administered in the diet to 25 presumed pregnant Sprague-Dawley (Crl:CD[SD]) rats per dose from GD 6 through LD 21 at a dose of 0, 40, 100 or 250 ppm (equal to 0, 3, 8 and 19 mg/kg bw per day during gestation and 0, 7, 16 and 40 mg/kg bw per day during lactation, respectively). The pups were not directly dosed. Dams were allowed to deliver naturally and were killed on LD 21. On PND 4, litters were randomly standardized to eight pups per litter (four of each sex where possible), and the remaining offspring were sacrificed without further examination. Subsequently, pups were allocated to Subset A, B or C. In Subset A (20 pups of each sex per dose group), the following parameters were examined: functional observational battery on PNDs 4, 11, 21, 35, 45 and 60; motor activity on PNDs 13, 17, 21 and 61; auditory startle response on PNDs 20 and 60; and learning and memory beginning on PND 62. Fifteen animals of each sex per dose group were selected from Subset A for brain weight evaluations on PND 72; of these, 10 animals of each sex from the control and 250 ppm groups were selected for neuropathological and morphometric evaluations on PND 72. All Subset A animals were also examined for balanopreputial separation or vaginal patency, as appropriate. For Subset B (20 pups of each sex per dose group), learning and memory testing was initiated on PND 25. For Subset C, 15 animals of each sex per dose group were selected for brain weight evaluations on PND 21; of these, 10 animals of each sex from the control and 250 ppm groups were selected for neuropathological and morphometric evaluations on PND 21.

A single female treated at 40 ppm died on LD 5, but the death was not considered treatment related. In the dams, there were no effects of treatment observed on mortality, body weight, body weight gain, feed consumption, reproductive parameters, gestation duration or macroscopic pathology. Treatment-related tremors were noted for 24 of 25 females treated at 250 ppm during lactation, with onset occurring between LDs 3 and 16. One female in this group also exhibited tremors on GD 13. The severity of the tremors generally increased as lactation progressed, consistent with the increased achieved intake of fenpropathrin on a milligram per kilogram body weight basis during lactation (40 mg/kg bw per day) compared with gestation (19 mg/kg bw per day). Modified functional observational battery assessments of the maternal animals on GD 10 revealed higher mean grooming counts for females treated at 250 ppm. At functional observational battery evaluation on LD 10, higher mean grooming counts and a higher number of females with slight tremors were noted in the 250 ppm group. In addition, one female in this group had moderately coarse tremors and slightly impaired mobility (LD 10) and splayed hindlimbs (LD 21). At the LD 21 evaluation, only one female in this group continued to exhibit slight tremors.

There were no effects of treatment on the number of pups born, sex ratio at birth, live litter size on PND 0, postnatal survival indices, the number of pups found dead, killed in extremis and/or missing or the general physical condition of the pups. Sexual developmental parameters (balanopreputial separation and vaginal patency) were also unaffected by maternal exposure.

During lactation, increased numbers of pups that were small in size were observed in the 250 ppm litters. This finding was often observed on multiple occasions in the affected pups and correlated with decreased pup body weights. Offspring preweaning body weights were decreased by 10–16% in both sexes from PNDs 7 through 21. Interval body weight gains for the corresponding intervals were also decreased by 11–21%. There were no effects of treatment on the functional observational battery, learning and memory, gross or microscopic pathology, or brain morphometric measurements.

Secondary neurobehavioural effects of the periweaning growth retardation observed in the 250 ppm group offspring included a tendency towards lessened habituation to the locomotor activity testing environment on PNDs 17 and 21. Test substance–related changes in acoustic startle response were observed in the 250 ppm group females on PND 60 relative to the control group.

Lower mean absolute brain weight and length were noted for F_1 male offspring on PND 21. Delayed brain development and thus smaller brains are seen in juvenile animals when caloric or nutrient restriction occurs during early postnatal development (Garman et al., 2001), and these effects may diminish or disappear completely in adults. The smaller brain weight and length in PND 21 males may indicate a test article–related effect on brain development, but an effect secondary to

nutrition cannot be ruled out, especially as final body and brain weights recovered by PND 72. Lower mean brain length in males was noted on PND 72. However, brain weights were similar to those in controls, no changes in mean brain width were noted and there were no test article–related behavioural effects noted during functional observational battery evaluations. From this weight of evidence, it was considered that the lower mean brain length in males was spurious and unrelated to test substance administration. The lower mean brain weight and length changes in males at PND 21 were considered probable test substance–related changes; however, the possibility that these changes were wholly or partially nutritionally induced cannot be discounted. Mean final body and brain weights recovered by PND 72.

The NOAEL for maternal toxicity was 100 ppm (equal to 8 mg/kg bw per day), based on tremors during lactation and effects on body weight and feed consumption at 250 ppm (equal to 19 mg/kg bw per day). The NOAEL for developmental neurotoxicity was 100 ppm (equal to 8 mg/kg bw per day), based on small size of pups and decreased body weights and body weight gains during the preweaning period, decreased habituation, increased mean overall maximum startle response amplitude and average response amplitude in the females, and decreased absolute brain weights in the males at 250 ppm (equal to 19 mg/kg bw per day) (Stump, 2008).

(d) Immunotoxicity

In an immunotoxicity study, fenpropathrin (purity 93%) was administered in the diet to female Crl:CD (SD) rats (10 per group) at a concentration of 0 (control), 150, 300 or 450 ppm for 28 consecutive days (days 0–28). Mean test substance consumptions were 0, 14, 26 and 42 mg/kg bw per day, respectively. On day 24, animals in all groups were immunized with a suspension of sheep red blood cells by intravenous injection (2×10^8 sheep red blood cells per animal, 0.5 ml/animal dose volume). A positive control group (10 females) received intraperitoneal injection of cyclophosphamide at a dose of 50 mg/kg bw (10 ml/kg bw) on study days 24–27. Animals were sacrificed on day 29. All animals were evaluated for mortality, clinical signs, body weight changes, feed consumption, water consumption, organ weights and gross pathology. Immunotoxicity was assessed for all animals by the antibody-forming cell (AFC) assay; spleen, thymus and adrenal gland weights were determined at necropsy. The dose levels were selected based on a range-finding study in which decreased body weights and body weight gains were seen at 450 ppm, but no treatment-related effects were observed at 150 and 300 ppm.

There were no effects of test substance treatment on mortality, water consumption, gross pathology or organ weights. Body weight gains were lower at 450 ppm during study days 0–3 and 7–10 compared with controls, and cumulative body weights were statistically significantly lower throughout the study except from study days 0 to 28. As a result, body weight in the 450 ppm group at study day 28 was 6.2% lower than that in the control group, although this was not statistically significant. Treatment-related clinical signs seen at 450 ppm were hyperactivity, hyperreactivity to touch, increased activity and twitching. There were no treatment-related effects on spleen cell number and no effect on the AFC response to the T cell–dependent antigen sheep red blood cells (i.e. specific activity and total spleen activity). A high interindividual variability was noted in all the treatment groups as well as in the control group. Evaluation of individual animal data did not show any trend or distribution that would demonstrate significant suppression of the anti–sheep red blood cell antibody response. Animals in the positive control group showed marked decreases in the anti–sheep red blood cell AFC response. The positive control group confirmed the validity of the immunotoxicity assay. A natural killer (NK) cell activity assay was not performed in this study.

The NOAEL for systemic toxicity was 300 ppm (equal to 26 mg/kg bw per day), based on lower body weight, reduced body weight gain and clinical signs at 450 ppm (equal to 42 mg/kg bw per day). The NOAEL for immunotoxicity was 450 ppm (equal to 42 mg/kg bw per day), the highest dose tested, based on an AFC assay for a T cell–dependent antibody response to immunization with sheep red blood cells. A LOAEL for immunotoxicity of fenpropathrin in female rats was not determined (Crittenden, 2011).

A series of studies by Stelzer & Gordon (1984a,b, 1985) showed that pyrethroids can affect the membrane lipid packing order of splenic lymphocytes and reported that fenpropathrin was able to inhibit mitogenic responsiveness of murine splenic lymphocytes to concanavalin A, thus suggesting possible concern for an immune suppression activity. However, the fully compliant immunotoxicity study, which includes evaluation of mitogenic responsiveness of splenic lymphocytes, submitted by the notifier demonstrated that the effects observed in vitro have no corroboration in vivo.

(e) Induction of hepatic microsomal enzymes

A preliminary investigation was performed to study the possible effect of fenpropathrin on the induction of hepatic microsomal enzymes. Four male Sprague-Dawley rats (one per dose) received fenpropathrin (purity 97%) in their diets at 1, 10, 100 or 1000 ppm over 2 weeks. Two positive controls received diets containing 100 ppm dieldrin. Liver homogenates were used to measure in vitro *O*-dealkylation of [^{14}C]chlorfenvinphos. The rate of *O*-deethylation of [^{14}C]chlorfenvinphos of fenpropathrin-treated rats was comparable to that of the controls. No dose-related increased in liver weights was observed. Rats treated with 100 ppm dieldrin showed a 16-fold increase in liver weights.

Therefore, the results of this study do not provide any evidence for an inducing effect of fenpropathrin on liver microsomal enzymes (Creedy & Potter, 1976).

(f) Study on antidotes

The therapeutic potency of intraperitoneally administered methocarbamol, or 3-(*o*-methoxy phenoxy)-1,2-propanediol 1-carbamate, was examined against acute oral intoxication of rats after treatment with lethal doses of fenpropathrin (100 mg/kg bw) and some other pyrethroids. Methocarbamol was initially administered intraperitoneally at a dose of 400 mg/kg bw, followed by repeated doses of 200 mg/kg bw. Treatment with methocarbamol reduced the mortality from 60% to 0% and depressed tremors (Hiromori et al., 1986b).

(g) Study on paraesthesia

To quantitatively evaluate the intensity of facial paraesthesia following exposure to fenpropathrin and other pyrethroids, the rabbit was used as the experimental model in a study performed in 1983. Groups of six rabbits were shaved 1 day before the study. A circle (2.54 cm in diameter) was marked at the centre. The animals were housed in well-illuminated cages, with a fan producing wind conditions (0.5 m/s) and allowing up and down observations through a mirror. The duration of licking and/or biting behaviour following application of 50 µl of the substance at different concentrations in acetone was recorded every 10 minutes for 2 hours. To evaluate the effectiveness of the treatment, additional animals were treated with 0.1 mg of the substance, and 400 mg of petroleum jelly–based 5% benzocaine ointment or undiluted vitamin E was applied within a 7.6 cm diameter circle, inclusive of the application site, 1 minute after application of pyrethroids.

The frequency of licking and/or biting after application showed a clear dose–response relationship with all pyrethroids tested. Post-treatment with petroleum jelly–based 5% benzocaine ointment (local anaesthetic) was effective in reducing the intensity of animal response. Post-treatment application of undiluted vitamin E was also effective for treatment of facial paraesthesia (Hiromori & Takemura, 1983).

(h) Endocrine effects

Andersen et al. (2002) tested 24 pesticides largely used in Denmark for interaction with the estrogen receptor and the androgen receptor in transactivation assays. Estrogen-like effects on MCF-7 cell proliferation and effects on cytochrome P450 19 aromatase activity in human placental microsomes were also investigated. Fenpropathrin was negative in all the assays.

3. Observations in humans

In a field study in Japan, six workers participated in two tests (four workers participating in both studies) using a 5% emulsifiable concentrate. The spray concentrations were 25–50 ppm (active ingredient), and the spraying time was 2 hours. A motor-mounted sprayer was used. The workers wore protective clothing. No effects were reported for all six subjective symptoms evaluated (i.e. headache, nasal discharge, itching, burning, pain in face or limbs) (Fujita, 1980).

In a paper by Matsumoto & Suzuki (no date), a case report describing facial sensation produced by fenpropathrin in 23 workers exposed to pyrethroids in the laboratory, during formulation processes or in field trials was discussed. As exposure to the different pyrethroids varied, it was not possible to determine which compounds were more likely to produce symptoms. It was concluded that fenpropathrin produces facial sensation in humans, and vitamin E and benzocaine proved to be effective therapeutics (Matsumoto & Suzuki, no date).

In a survey of 18 operators using a 10% emulsifiable concentrate fenpropathrin formulation, no adverse effects were reported, with the exception of slight nasal irritation (single report). All operators wore protective clothing (Shell Chemical Ltd, 1987).

Fourteen workers were engaged in fenpropathrin manufacturing at Sumitomo Chemical's Oita Works for the previous decade (2002–2011). Production volume ranged between 50 and 220 tonnes in each fiscal year. Periodic medical examinations were carried out at least once per year on each worker, according to the Japanese requirements. The medical examination included investigation of medical history and work habits, smoking, body measurements, eyesight and hearing, chest X-ray examination, blood pressure measurement, haematology measurements, liver function tests, blood lipid examination, blood sugar, renal function tests, urine analysis and electrocardiogram examination. No adverse effects were reported by the engaged workers, and no health problems or adverse findings were noted at the periodic examinations (Nishioka, 2011).

Comments

Biochemical aspects

Absorption of fenpropathrin following a single oral administration was rapid, and elimination was almost complete (about 57% in urine and about 40% in faeces) within 48 hours. Low concentrations of residues (< 0.6 µg/g tissue) were measured in blood, liver, kidney, fat, muscle and brain within 24 hours after dosing, and concentrations declined rapidly for 8 days, except for those in fat, which were the highest concentrations measured and which also declined, but not as rapidly. Less than 1.5% of the administered dose remained in the body 8 days after treatment.

The major biotransformation reactions of fenpropathrin in rats consisted of oxidation at the methyl groups of the acid moiety and at the 2′- and 4′-positions of the alcohol moiety, cleavage of the ester linkage and conjugation of the resultant carboxylic acids, alcohols and phenols with glucuronic acid, sulfuric acid and glycine. Most of the urinary metabolites were ester-cleaved products. The predominant urinary metabolites derived from the acid moiety were identified as TMPA–glucuronide and TMPA-CH_2OH (*trans*). The major urinary metabolites derived from the alcohol moiety were PBacid in free form and as glycine conjugate, 4′-OH-PBacid–sulfate and 2′-OH-PBacid–sulfate. The major faecal metabolite was identified as CH_2OH *trans*-fenpropathrin, followed by COOH *trans*-fenpropathrin, 4′-OH-fenpropathrin and 4′-OH,CH_2OH *trans*-fenpropathrin. Depending on the dose administered, 30–50% of the applied radioactivity was excreted in faeces as parent compound. Fenpropathrin and TMPA were the major radiolabelled compounds in tissues. An aryl-hydroxylated ester (α-cyano-3-(4-hydroxyphenoxy)benzyl ester) was identified in bile.

Toxicological data

Fenpropathrin appears to have both Type I and Type II properties. It produces repetitive firing of neurons but is associated with Type II symptoms. In acute studies with fenpropathrin in mammals, onset of toxic signs is rapid (within a few hours or days), independent of the route of exposure.

Recovery of surviving animals is also rapid. Toxic signs are those typical for pyrethroids and include hypersensitivity, fibrillation, tremors, clonic convulsions, salivation, lacrimation, urinary incontinence and hindlimb and/or whole-body ataxia. The acute oral LD_{50} in rats is greater than or equal to 48.5 mg/kg bw, depending on the vehicle. The dermal LD_{50} in a study in rats ranged from 870 mg/kg bw to greater than 5000 mg/kg bw, depending on the vehicle. The acute inhalation LC_{50} in rats was greater than or equal to 556 mg/m^3. Fenpropathrin is a slight skin irritant and is minimally irritating to the eyes of rabbits. It is not a dermal sensitizer in the Buehler test.

The neurological clinical signs (body tremors, hypersensitivity/hyperreactivity, ataxia and, in dogs only, emesis) and reduced body weight gain are the key and most sensitive toxicological end-points.

In a 28-day toxicity study in mice, the NOAEL was 500 ppm (equal to 63 mg/kg bw per day), based on decreases in body weight gain in males, decreases in feed efficiency in males and clinical signs seen at 1000 ppm (equal to 123 mg/kg bw per day).

In three 90-day studies of toxicity in rats, the overall NOAEL was 450 ppm (equal to 21.3 mg/kg bw per day), based on decreased body weight gain, decreased feed consumption and slightly increased alkaline phosphatase activity seen at 600 ppm (equal to 28.8 mg/kg bw per day).

In a 1-year dietary study of toxicity in dogs, the NOAEL was 100 ppm (equal to 3.1 mg/kg bw per day), based on reduced body weight gain and clinical signs (emesis, tremors) at 250 ppm (equal to 7.7 mg/kg bw per day). Similar toxic effects were observed at 250 ppm (equal to 7.4 mg/kg bw per day), the lowest dose tested, in a 90-day study of toxicity in dogs.

A 2-year toxicity and carcinogenicity study was performed in mice in which the NOAEL was 600 ppm (equal to 56 mg/kg bw per day), the highest dose tested. No evidence of carcinogenicity was observed. In a second study of carcinogenicity in mice, a dose of 1000 ppm caused the death of 38% of the males and 15% of the females within 13 weeks, indicating a steep toxicity–response curve and permitting the conclusion that the first study, in which the highest dose tested was 600 ppm (equal to 56 mg/kg bw per day), the maximum achievable dose, was adequate for an assessment of the carcinogenicity of this compound.

Two long-term toxicity and carcinogenicity studies in rats were available. The overall NOAEL was 150 ppm (equal to 7.1 mg/kg bw per day), based on depression in body weight gain and clinical signs at 450 ppm (equal to 21.9 mg/kg bw per day). There was no evidence of carcinogenicity.

The Meeting concluded that fenpropathrin was not carcinogenic in mice or rats.

Fenpropathrin was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. In none of these assays was there any evidence of genotoxic potential.

The Meeting concluded that fenpropathrin was unlikely to be genotoxic.

On the basis of the lack of genotoxicity and the absence of carcinogenicity in mice and rats, the Meeting concluded that fenpropathrin is unlikely to pose a carcinogenic risk to humans.

Two multigeneration reproductive studies are available in rats. The overall NOAEL for parental systemic toxicity was 40 ppm (equal to 2.6 mg/kg bw per day), based on depression of body weight gain, increased mortality in females and the occurrence of body tremors and muscle twitches at 120 ppm (equal to 7.8 mg/kg bw per day). No effects on reproductive parameters were observed at doses up to 360 ppm (equal to 23.3 mg/kg bw per day), the highest dose tested. The overall NOAEL for offspring toxicity was 40 ppm (equal to 2.6 mg/kg bw per day), based on body tremors seen in some pups at 120 ppm (equal to 7.8 mg/kg bw per day).

Two studies of developmental toxicity in rats were available. The overall NOAEL for maternal toxicity was 3 mg/kg bw per day, based on reduced feed consumption and body weight gain at the beginning of treatment seen at 6 mg/kg bw per day. The NOAEL for developmental toxicity was 10 mg/kg bw per day, the highest dose tested.

Developmental toxicity studies were conducted with rabbits. The overall NOAEL for maternal toxicity was 4 mg/kg bw per day, based on clinical signs noted at 12 mg/kg bw per day, whereas the overall developmental NOAEL was 36 mg/kg bw per day, the highest dose tested.

The Meeting concluded that fenpropathrin is not teratogenic in rats or rabbits.

In an acute neurotoxicity study, the NOAEL was 10 mg/kg bw, based on tremors at 25 mg/kg bw. In a separate study, no histopathology of sciatic and tibial nerves or increase in β-glucuronidase activity (indicative of Wallerian degeneration in nerves) was observed in rats at doses up to 500 ppm (equivalent to 25 mg/kg bw per day).

In a published study, effects on motor activity were measured for several pyrethroids, including fenpropathrin, following administration of a single gavage dose in corn oil to rats. Threshold doses (calculated dose at which treated rats did not display any decreases in motor activity) were calculated for these pyrethroids. The threshold dose for fenpropathrin was 3.06 mg/kg bw. The ED_{30} for fenpropathrin was 7.70 ± 0.65 mg/kg bw. In an acute neurotoxicity study in rats, the NOAEL was 15 mg/kg bw, based on slight tremors and clonic convulsions (whole-body tremors) in both sexes at the time of peak effect seen at the LOAEL of 30 mg/kg bw.

In a 90-day neurotoxicity study, treatment at 570 ppm caused the death of 1 of 12 females, decreased body weight gain and feed consumption in both sexes, and led to clinical signs and a number of alterations in functional observational batteries. At 190 ppm (equal to 15 mg/kg bw per day for females), walking on tiptoes and hunched body were observed during the open-field observations in females only. The NOAEL was 60 ppm (equal to 5 mg/kg bw per day for females).

The range-finding study for a guideline developmental neurotoxicity study in rats confirmed the presence of fenpropathrin in milk of lactating females as well as in plasma of mothers and pups, demonstrating that the dietary route of exposure was valid for the main developmental neurotoxicity study. The LOAEL for developmental neurotoxicity was 250 ppm (equal to 19 mg/kg bw per day), based on small pups and decreased body weights and body weight gains during the preweaning period, decreased habituation, increased mean overall maximum startle response amplitude and average response amplitude in the females, and decreased absolute brain weights in the males. The NOAEL for developmental neurotoxicity was 100 ppm (equal to 8 mg/kg bw per day).

No signs of neurotoxicity and no histopathological findings were observed in the nervous system of hens treated with fenpropathrin at 1000 mg/kg bw per day.

No immunotoxic potential for fenpropathrin was evidenced in a specific study in which rats were administered up to 450 ppm (42 mg/kg bw per day), a dose level causing systemic toxicity.

No adverse effects were reported by 14 workers engaged in fenpropathrin manufacturing (2002–2011), and no health problems or adverse findings were noted at periodic examinations.

The Meeting concluded that the existing database on fenpropathrin was adequate to characterize the potential hazards to fetuses, infants and children.

Toxicological evaluation

The Meeting reaffirmed the ADI of 0–0.03 mg/kg bw on the basis of an overall NOAEL of 100 ppm (equal to 3.1 mg/kg bw per day) in the 90-day and 1-year toxicity studies in dogs, based on the occurrence of tremors seen at 250 ppm (equal to 7.4 mg/kg bw per day), and using a safety factor of 100. This ADI was supported by the NOAEL of 40 ppm (equal to 2.6 mg/kg bw per day) observed in a multigeneration reproductive study in rats, on the basis of the occurrence of body tremors and muscle twitches and mortality of two females seen at 120 ppm (equal to 7.8 mg/kg bw per day). It is further supported by the combined NOAEL of 3 mg/kg bw per day observed in the developmental toxicity studies in rats, on the basis of decreases in body weight gain and feed consumption seen at 6 mg/kg bw per day.

The Meeting established an ARfD of 0.03 mg/kg bw on the basis of the threshold dose of 3.06 mg/kg bw from a published study measuring motor activity at the time of peak effects following a single oral dose in rats and using a safety factor of 100. This ARfD value was supported by the combined NOAEL of 3 mg/kg bw per day seen in the developmental toxicity studies in rats, based on a decrease in body weight gain and feed consumption in dams during the first 2 days of dosing at 6 mg/kg bw per day.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year study of toxicity and carcinogenicity[a]	Toxicity	600 ppm, equal to 56 mg/kg bw per day[b]	—
		Carcinogenicity	600 ppm, equal to 56 mg/kg bw per day[b]	—
Rat	Ninety-day studies of toxicity[a,c]	Toxicity	450 ppm, equal to 21.3 mg/kg bw per day	600 ppm, equal to 28.8 mg/kg bw per day
	Two-year studies of toxicity and carcinogenicity[a,c]	Toxicity	150 ppm, equal to 7.1 mg/kg bw per day	450 ppm, equal to 21.9 mg/kg bw per day
		Carcinogenicity	600 ppm, equal to 22.7 mg/kg bw per day[b]	—
	Multigeneration studies of reproductive toxicity[a,c]	Reproductive toxicity	360 ppm, equal to 23.3 mg/kg bw per day[b]	—
		Parental toxicity	40 ppm, equal to 2.6 mg/kg bw per day	120 ppm, equal to 7.8 mg/kg bw per day
		Offspring toxicity	40 ppm, equal to 2.6 mg/kg bw per day	120 ppm, equal to 7.8 mg/kg bw per day
	Developmental toxicity studies[c,d]	Maternal toxicity	3 mg/kg bw per day	6 mg/kg bw per day
		Embryo and fetal toxicity	10 mg/kg bw per day[b]	—
	Acute neurotoxicity study[d] (published)	Neurotoxicity	Threshold dose: 3.06 mg/kg bw	Estimated ED_{30}: 7.70 mg/kg bw
	Ninety-day study of neurotoxicity[a]	Toxicity	60 ppm, equal to 5 mg/kg bw per day	190 ppm, equal to 15 mg/kg bw per day
Rabbit	Developmental toxicity studies[c,d]	Maternal toxicity	4 mg/kg bw per day	12 mg/kg bw per day
		Embryo and fetal toxicity	36 mg/kg bw per day[b]	—
Dog	Ninety-day and 1-year studies of toxicity[a,c]	Toxicity	100 ppm, equal to 3.1 mg/kg bw per day	250 ppm, equal to 7.4 mg/kg bw per day

ED_{30}, dose (mg/kg bw) required to induce a 30% decrease in total motor activity compared with the corresponding vehicle-treated control group

[a] Dietary administration.

[b] Highest dose tested.

[c] Two or more studies combined.

[d] Gavage administration.

Estimate of acceptable daily intake for humans

0–0.03 mg/kg bw

Estimate of acute reference dose

0.03 mg/kg bw

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposures

Critical end-points for setting guidance values for exposure to fenpropathrin

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid, at least 57%
Dermal absorption	Not available
Distribution	Widely distributed
Potential for accumulation	No
Rate and extent of excretion	Rapid and complete
Metabolism in animals	Extensive
Toxicologically significant compounds in animals, plants and the environment	Parent compound
Acute toxicity	
Rat, LD_{50}, oral	≥ 48.5 mg/kg bw (vehicle dependent)
Rat, LD_{50}, dermal	≥ 870 mg/kg bw (vehicle dependent)
Rat, LC_{50}, inhalation	≥ 556 mg/m^3 (nose-only exposure)
Rabbit, dermal irritation	Slightly irritating
Rabbit, ocular irritation	Mildly irritating
Dermal sensitization	Non-sensitizing, Buehler test
Short-term studies of toxicity	
Target/critical effect	Neurotoxic signs
Lowest relevant oral NOAEL	3.1 mg/kg bw per day (dog)
Lowest relevant dermal NOAEL	1000 mg/kg bw per day (rat)
Lowest relevant inhalation NOAEC	Not available
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Neurotoxic signs
Lowest relevant oral NOAEL	Combined 7.1 mg/kg bw per day (rat)
Carcinogenicity	Not carcinogenic
Genotoxicity	
	Not genotoxic
Reproductive toxicity	
Target/critical effect	Neurotoxic signs
Lowest relevant parental NOAEL	2.6 mg/kg bw per day
Lowest relevant offspring NOAEL	2.6 mg/kg bw per day

Lowest relevant reproductive NOAEL	23.3 mg/kg bw per day (highest dose tested)
Developmental toxicity	
Developmental target/critical effect	Decreased body weight gain and secondary effects on locomotor activity
Lowest maternal NOAEL	3 mg/kg bw per day
Lowest embryo/fetal NOAEL	10 mg/kg bw per day
Neurotoxicity/delayed neurotoxicity	
Acute neurotoxicity NOAEL	3 mg/kg bw
Subchronic neurotoxicity NOAEL	5 mg/kg bw per day
Developmental neurotoxicity NOAEL	8 mg/kg bw per day
Delayed neurotoxicity	No signs of delayed neurotoxicity
Immunotoxicity	
Immunotoxicity NOAEL	42 mg/kg bw per day (highest dose tested)
Medical data	
	No adverse health effects reported in manufacturing plant personnel

Summary

	Value	Study	Safety factor
ADI	0–0.03 mg/kg bw	Ninety-day and 1-year toxicity studies (dog)	100
ARfD	0.03 mg/kg bw	Single-dose neurotoxicity study (rat)	100

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FENVALERATE

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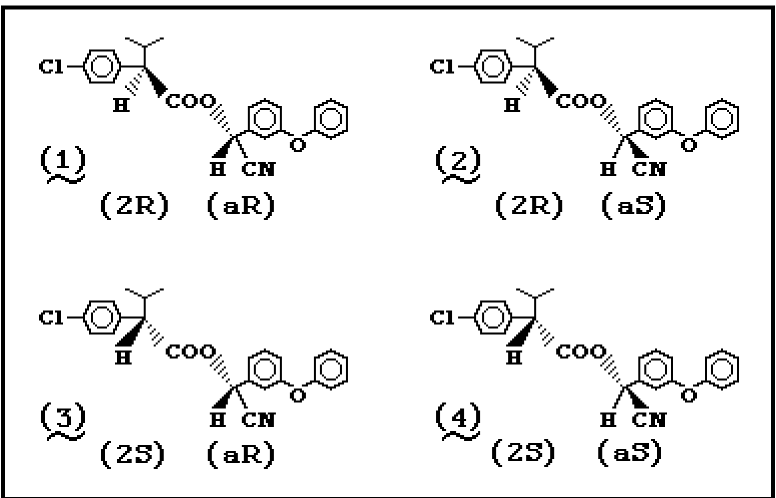
Explanation

Fenvalerate is the International Organization for Standardization (ISO)–approved common name for (*RS*)-α-cyano-3-phenoxybenzyl (*RS*)-2-(4-chlorophenyl)-3-methylbutyrate (International Union of Pure and Applied Chemistry), for which the Chemical Abstracts Service number is 51630-58-1. It is a broad-spectrum pyrethroid with neurotoxic effects on insect pests.

Fenvalerate (Figure 1) is a racemic mixture of four stereoisomers ([2*S*,α*S*], [2*S*,α*R*], [2*R*,α*S*] and [2*R*,α*R*]) (Figure 2) found in approximately equal proportions owing to the presence of two chiral centres. One of these four chiral isomers, esfenvalerate, is the [2*S*,α*S*] or A-α isomer and has been developed separately in the knowledge that it was the biologically active component of the fenvalerate racemic mixture. Fenvalerate is classified, according to its structure, as a type II pyrethroid.

Figure 1. Chemical structure of fenvalerate

Figure 2. Chemical structure of the four stereoisomers of fenvalerate



Fenvalerate was reviewed by the Joint FAO/WHO Meeting on Pesticide Residues on four previous occasions. Temporary acceptable daily intakes (ADIs) of 0–0.06, 0–0.007 and 0–0.02 mg/kg body weight (bw) were established by the Meeting in 1979, 1981 and 1984, respectively, and an ADI of 0–0.02 mg/kg bw was established by the Meeting in 1986.

Fenvalerate is being reviewed by the present Meeting at the request of the Codex Committee on Pesticide Residues.

As the compound was not supported by a company that would provide toxicological studies for review, access to the toxicological studies for the current evaluation was provided by the United States Environmental Protection Agency (USEPA). The evaluation of fenvalerate was based on the previous reviews by JMPR (Annex 1, references 32, 33, 36, 37, 42, 43, 47 and 49), an International Programme on Chemical Safety evaluation (IPCS, 1990), the esfenvalerate review by JMPR (Annex 1, references 95 and 97), studies submitted to the USEPA and published studies from the open literature.

Most of the studies do not comply with good laboratory practice (GLP), as most of the data were generated before the implementation of GLP regulations. Overall, the Meeting considered that the database was adequate for the risk assessment.

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

Mice

Five male and five female ddY mice were administered [^{14}C-chlorophenyl]- or [^{14}C-phenoxybenzyl]fenvalerate (2.5 or 10 mg/kg bw), [^{14}C-chlorophenyl]- or [^{14}C-phenoxybenzyl]esfenvalerate (2.5 mg/kg bw) or [^{14}C-chlorophenyl]- or [^{14}C-phenoxybenzyl]esfenvalerate mixed with the three other unlabelled isomers. The chemicals were administered orally by gavage in corn oil. Urine and faeces were collected daily for 7 days. The animals were killed 7 days after dosing, and major tissues were excised. Excreta and tissues were analysed for radioactivity by LSC. In both sexes, 86–94% of the administered radioactivity was excreted within 1 day of dosing, and 94–102% was excreted within 7 days. Roughly equal quantities of radioactivity were excreted in the urine and faeces. The highest residue levels were detected in the fat tissues, with 0.118–0.484 µg equivalents per gram following a dose of 2.5 mg/kg bw and 1.16–1.579 µg equivalents per gram following a dose of 10 mg/kg bw (Kaneko et al., 1985).

In a 28-day dietary study, ddY mice were fed [^{14}C-chlorophenyl]esfenvalerate at a level of 25 parts per million (ppm) or [^{14}C-chlorophenyl]fenvalerate at a level of 25 or 100 ppm. The estimated daily intakes were 115–119 µg/mouse from the 25 ppm esfenvalerate diet and 112–118 µg/mouse and 424–477 µg/mouse from the 25 and 100 ppm fenvalerate diets, respectively. Groups of six male and six female mice were killed after feeding on the treated diets for 10, 19, 24 and 28 days and subsequently feeding on untreated diets for 4, 7, 21 and 28 days after termination of the 28-day treatment period. Concentrations of residues were highest in fat (about 7 µg esfenvalerate equivalents per gram tissue after 24–28 days on the 25 ppm esfenvalerate diet), about 7-fold lower in the adrenal gland, lymph node and skin, about 10-fold lower in liver and about 25-fold lower in blood. Levels of radioactivity approached a plateau after 24–28 days of feeding and subsequently declined when mice were returned to untreated diet. Total radioactivity concentrations in some tissues (adrenals, spleen and ovaries) were lower following feeding with esfenvalerate than with a similar dose level of fenvalerate. In other tissues, the levels of total radioactivity were similar. Total radioactivity was approximately 4 times higher in the tissues of animals fed fenvalerate at 100 ppm in the diet than in the tissues of animals fed fenvalerate at 25 ppm in their diet. There were no differences between the sexes (Isobe et al., 1985).

Groups of five male and five female ddY mice received 10 consecutive daily oral doses of [^{14}C-chlorophenyl]esfenvalerate at 2.5 mg/kg bw or [^{14}C-chlorophenyl]fenvalerate at 10 mg/kg bw. Excreta were collected daily for 7 days after the last dose. Most of the radioactivity (90% of that administered) was excreted within 1 day after the last dose. Excretion was 91–98% complete within 7 days, with equal amounts found in the urine and faeces. The study demonstrated that repeated dosing of either esfenvalerate or fenvalerate did not alter the excretion patterns with respect to dose, sex or species (Kaneko et al., 1985).

Mice and rats

A study was conducted in which ddY mice (four males and four females) and Sprague-Dawley rats (one male and one female) were given single oral doses of [^{14}C-carbonyl]-, [^{14}C-benzylic]- or [^{14}C-cyano]fenvalerate at 7.0 mg/kg bw or [^{14}C-chlorophenyl]-, [^{14}C-phenoxybenzyl]- or [^{14}C-cyano]esfenvalerate at 4.2 mg/kg bw. Each of the ^{14}C-labelled fenvalerates was also administered to two male and two female rats at the rate of 30 mg/kg bw. Urine and faeces were collected daily for 6–7 days, and carbon dioxide was collected from those mice and rats that received ^{14}C-cyano-labelled compound. The mice and rats were killed 6–7 days after dosing. Excreta and tissues were analysed for

metabolites by thin-layer chromatography (TLC), autoradiography and liquid scintillation counting (LSC).

The half-life for excretion in both rodent species was 0.5–0.6 day. Elimination of the ^{14}C-cyano-labelled fenvalerate was somewhat slower in both species, suggesting a different pattern of metabolism. Total recovery of the administered ^{14}C-cyano-labelled fenvalerate was achieved within 6 days following the acute administration. Tissue residues following acute administration were extremely low, with the highest concentrations being observed in fat, adrenal gland, skin, hair and intestines. High concentrations of the ^{14}C-cyano-labelled fenvalerate were noted in the hair and skin, which may account for the data showing that residues of this label were more slowly excreted from the body (Kaneko, Ohkawa & Miyamoto, 1979; Ohkawa et al., 1979).

Groups of four male and four female ddY mice and Sprague-Dawley rats were administered a single oral gavage dose of [^{14}C-carbonyl]fenvalerate and [^{14}C-benzylic]fenvalerate. The dose level was 6.7–7.8 mg/kg bw and 30 mg/kg bw for mice and rats, respectively. Urine and faeces were collected at several intervals for 6 or 7 days. The total recovery of the radioactivity 6 or 7 days after administration was 93–102% in mice and rats. The half-life of the radioactivity was 0.5–0.6 day in both species. In rats dosed with [^{14}C-chlorophenyl]- or [^{14}C-phenoxybenzyl]fenvalerate at 30 mg/kg bw, excretion in the urine and faeces was 86–97% complete in 6 days, with a half-life of 0.6–0.9 day. In rats given low doses of [^{14}C-cyano]fenvalerate and esfenvalerate, excretion of the radioactivity was 75–81% complete after 6–7 days, with a half-life of 1.7–2.0 days. In similarly treated mice, excretion was 88–89% complete by 6–7 days, with a half-life of 1–1.7 days. Exhaled $^{14}CO_2$ accounted for 6–14% of the administered radioactivity in both species. Tissue residues in mice and rats given low doses of [^{14}C-chlorophenyl]- and [^{14}C-phenoxybenzyl]esfenvalerate and [^{14}C-carbonyl]- and [^{14}C-benzylic]fenvalerate were very low 6–7 days after dosing. The highest levels were in fat, ranging from 0.45 to 0.89 mg equivalents per kilogram in mice and from 0.71 to 1.5 mg equivalents per kilogram in rats. When fenvalerate at 30 mg/kg bw was dosed to rats, residues in fat were 4.3–8.9 mg equivalents per kilogram. In blood, hair, liver and skin of both species, the levels were less than 0.36 mg equivalents per kilogram at the low dose level and 0.81 mg equivalents per kilogram at the high dose level. However, ^{14}C-cyano-labelled compounds produced higher residues in hair, skin and stomach contents (see Ohkawa et al., 1979, above) (Kaneko, Ohkawa & Miyamoto, 1981).

Rats

Single oral doses (20 µmol/kg bw) or five daily doses (4 µmol/kg bw per day) of [^{14}C-cyano]fenvalerate, [^{14}C-cyano][2*S*,α*RS*]-fenvalerate, [^{14}C-benzylic][2*S*,α*RS*]-fenvalerate, [^{14}C-carbonyl][2*S*,α*RS*]-fenvalerate, [^{14}C-carbonyl]2-(4-chlorophenyl)isovaleric acid (CPIA), KS[^{14}CN] or K[^{14}CN] suspended in 10% Tween 80 were administered to male Sprague-Dawley rats. Urine, faeces and expired carbon dioxide were collected daily for 6 and 14 days after administration of a single dose and for 6 days after the last of five daily doses. Tissue samples were obtained 6 and 14 days after dosing. Samples were analysed by TLC, autoradiography and LSC.

Similar excretion patterns were seen with single and repeated administration, except for KS[^{14}CN] and K[^{14}CN] (Tables 1 and 2). The administered radioactivity was almost completely recovered from the urine and faeces within 2 days of dosing with [^{14}C-carbonyl]- and [^{14}C-benzylic][2*S*,α*RS*]-fenvalerate (92.5% and 93.5%, respectively). None of the radioactivity administered as fenvalerate or as [2*S*,α*RS*]-fenvalerate was excreted as $^{14}CO_2$. Slower excretion was seen with KS[^{14}CN] and K[^{14}CN], of which small amounts were excreted as carbon dioxide in expired air (15.0% and 7.5%, respectively). Tissue residues resulting from dosing with ^{14}C-carbonyl- or ^{14}C-benzylic-labelled [2*S*,α*RS*]-fenvalerate were generally 0.3 mg equivalents per kilogram or less 6 days after a single dose and declined to 0.1 mg equivalents per kilogram by 14 days after dosing in adrenals, blood, brain, caecum, hair, heart, intestine, kidney, liver, lung, muscle, pancreas, sciatic nerve, skin, spinal cord, spleen, stomach and testis. The only exception was fat, which contained 2 mg equivalents per kilogram 6 days after dosing with [^{14}C-carbonyl][2*S*,α*RS*]-fenvalerate. These residues declined to 0.5 mg equivalents per kilogram 14 days after dosing.

Table 1. Percentage of administered radioactivity excreted 6 days after a single oral dose (20 µmol/kg bw) in rats

	% of administered radioactivity						
	[^{14}C-carbonyl]-[2*S*,α*RS*]-Fenvalerate	[^{14}C-benzylic]-[2*S*, α*RS*]-Fenvalerate	[^{14}C-cyano]-[2*S*,α*RS*]-Fenvalerate	[^{14}C-cyano]-Fenvalerate	[^{14}C-carbonyl]-CPIA	K[^{14}CN]	KS[^{14}CN]
Expired air	0	0	7.5	10	0	7.5	15.0
Urine	60.0	45.5	21.5	23.75	77.5	32.5	31.25
Faeces	32.5	48.0	37.5	41.25	20.0	22.5	12.50
Total	92.5	93.5	66.5	75	97.5	62.5	58.75

From Ohkawa et al. (1979)
CPIA, 2-(4-chlorophenyl)isovaleric acid

Table 2. Percentage of administered radioactivity excreted 6 days after five consecutive daily doses of [2S,αRS]-fenvalerate (4 µmol/kg bw per day) in rats

	% of administered radioactivity		
	Carbonyl	Benzylic	Cyano
Expired air	0	0	7
Urine	47.5	58.75	25
Faeces	50.0	37.50	33.75
Total	97.5	96.25	63.75

From Ohkawa et al. (1979)

For [^{14}C-cyano]fenvalerate, [^{14}C-cyano][2*S*,α*RS*]-fenvalerate, K[^{14}CN] and KS[^{14}CN] administered as a single oral dose of 20 µmol/kg bw, the highest residue levels were found in hair and skin. Hair contained 36 mg [2*S*,α*RS*]-fenvalerate equivalents per kilogram, 41 mg KCN equivalents per kilogram and 119 mg KSCN equivalents per kilogram 6 days after dosing. At the same time point, skin contained 3 mg [2*S*,α*RS*]-fenvalerate equivalents per kilogram, 4.5 mg KCN equivalents per kilogram and 9 mg KSCN equivalents per kilogram. Fat and blood contained 1.2–1.4 mg [2*S*,α*RS*]-fenvalerate or fenvalerate equivalents per kilogram, and blood contained 1.4–1.7 mg KSCN or KCN equivalents per kilogram. A similar pattern was seen after repeated dosing.

Autoradiography showed that 6 and 24 hours after a single oral dose (20 µmol/kg bw) of [^{14}C-cyano][2*S*,α*RS*]-fenvalerate, radioactivity was distributed throughout the body, except to the brain and spinal cord. The highest concentrations (after the gastrointestinal tract) appeared to be in liver and lung, but this estimation is only as a result of visual inspection of the single autoradiograph presented 6 and 24 hours after dosing. At 144 hours after dosing, radioactivity was detected only in the hair, skin and stomach contents (the last, presumably, as a result of grooming). The distribution to the skin did not appear to show regional variation, which might have indicated that it was the result of contamination from urine. Six days after a single dose of [^{14}C-cyano][2*S*,α*RS*]-fenvalerate, TLC analysis of the stomach contents showed that essentially all of the radioactivity was due to thiocyanate at a concentration of 41 ppm. A similar pattern was seen with KS[^{14}CN] and K[^{14}CN]. Concentrations of [2*S*,α*RS*]-fenvalerate in blood and liver reached maximum levels (0.5 mg [2*S*,α*RS*]-fenvalerate equivalents per kilogram) 3 hours after a single dose of [^{14}C-cyano][2*S*,α*RS*]-fenvalerate and then rapidly declined to about 0.01 mg [2*S*,α*RS*]-fenvalerate equivalents per kilogram after 48 hours. There

appeared to be no significant differences in the excretion patterns of [2*S*,α*RS*]-fenvalerate and fenvalerate (Ohkawa et al., 1979).

Male and female rats fed fenvalerate (20 ppm) for 28 days and placed on control diets for an additional 28 days were examined for tissue residues and their depletion rates. Maximum residues were reached rapidly, within 3 weeks of dietary administration. Of the tissues measured, adipose tissue contained the highest residue. Trace amounts were observed in other tissues, including the brain, after 28 days of treatment. Dissipation of residues from all tissues following the cessation of treatment was rapid, although the dissipation was slower with adipose tissue than with other tissues. At 28 days after the cessation of administration of dietary fenvalerate, residues were still reported in adipose tissue, attesting to the slow clearance from this storage depot (Potter, 1976; Potter & Arnold, 1977).

In a separate study, fenvalerate was shown to dissipate from the adipose tissue of Sprague-Dawley rats, dosed orally with either [2*RS*,α*RS*]- or [2*S*,α*S*]-fenvalerate at 3 mg/kg bw, with a half-life of about 7 days in each case. The pyrethroid level in the brain of rats treated intraperitoneally with [2S,αS]-fenvalerate at 2.5 mg/kg bw dissipated with a half-life of about 2 days (Marei, Ruzo & Casida, 1982).

Five male and five female Sprague-Dawley rats were administered [^{14}C-chlorophenyl]- or [^{14}C-phenoxybenzyl]fenvalerate (2.5 or 10 mg/kg bw), [^{14}C-chlorophenyl]- or [^{14}C-phenoxy-benzyl]esfenvalerate (2.5 mg/kg bw) or [^{14}C-chlorophenyl]- or [^{14}C-phenoxybenzyl]esfenvalerate mixed with the three other unlabelled isomers. The chemicals were administered orally by gavage in corn oil. Urine and faeces were collected daily for 7 days. The rats were killed 7 days after dosing, and major tissues were excised. Excreta and tissues were analysed for radioactivity by LSC. There were no significant differences between dose levels, sexes or quantities of metabolites in the excretion of ^{14}C-chlorophenyl- and ^{14}C-phenoxybenzyl-labelled material. Approximately 63–86% of the radioactivity was excreted 1 day after dosing, and 95–101% had been excreted by 7 days after dosing. Between 20% and 39% of the radioactivity was excreted in the urine, with the remainder in the faeces. Tissue levels were generally very low, with the highest residues found in fat (180–310 µg equivalents per kilogram for the low-dose groups and 1320 µg equivalents per kilogram for the high-dose group). Residue levels from the fenvalerate treatment (10 mg/kg bw) were generally 4 times higher than the residues from the esfenvalerate treatment (2.5 mg/kg bw) (Kaneko et al., 1985).

A placental transfer study was conducted in Sprague-Dawley rats. Groups of three pregnant rats were dosed orally with [^{14}C-chlorophenyl]esfenvalerate at 2.5 mg/kg bw per day or [^{14}C-chlorophenyl]fenvalerate at 10 mg/kg bw per day in corn oil. Dosing began on day 13 of gestation and consisted of either a single dose of ^{14}C-labelled compound or three consecutive daily doses of unlabelled compound followed by two consecutive daily doses of ^{14}C-labelled compound. The rats were killed 3, 6, 12, 24 or 48 hours after the final dose. The fetuses, amniotic fluid, placentas, maternal blood and ovaries were examined for radioactivity using LSC. Maximum levels of radioactivity were found within 12 hours after the last dose, after which they declined rapidly. Fenvalerate residues were generally 4 times higher than esfenvalerate residues, except in maternal blood, placenta, amniotic fluid and ovaries 24 hours after a single dose, where the difference was 18–31 times higher, and the ovaries 24 and 48 hours after multiple doses, where the levels were similar. Tissue levels were at their peak 3–6 hours after the last dose and then declined rapidly. The highest levels of residues from both pyrethroids were found in maternal blood and ovaries, and the lowest were found in the fetuses and amniotic fluid. The highest residue level found in the fetuses was 152 µg esfenvalerate equivalents per kilogram, 6 hours after the last of five consecutive daily doses. Over the whole experiment, less than 0.07% of the applied radioactivity was found in the fetuses, indicating

that there was virtually no transfer of radioactivity from maternal blood to the fetuses. There was no evidence of accumulation of esfenvalerate in fetal tissues or amniotic fluid of rats (Shiba et al., 1985).

After administration of a single oral dose or five consecutive oral doses of [^{14}C]esfenvalerate or [^{14}C]fenvalerate labelled in the acid moiety to 13-day pregnant rats at a rate of 2.5 or 10 mg/kg bw per day, respectively, the maternal blood and placenta generally showed higher ^{14}C levels as compared with the fetus and amniotic fluid. Both compounds did not transfer readily from the maternal blood to the fetus, the amount of ^{14}C transferred being less than 0.07% of the dose. There were no substantial differences in the fetal ^{14}C level or the transfer ratio (^{14}C tissue level/^{14}C maternal blood level) between both labelled preparations. Overall, esfenvalerate and fenvalerate seemed to behave in the same manner as far as placental transfer was concerned (Shiba et al., 1990).

Dogs

The metabolism of fenvalerate was studied in dogs using [^{14}C-chlorophenyl]- and [^{14}C-phenoxybenzyl]fenvalerate. A single oral dose (1.7 mg/kg bw), dissolved in corn oil, was administered in gelatine capsules. Excreta and blood were collected daily for 3 days and analysed for radioactivity by LSC. Metabolites were analysed using TLC using four solvent systems. Elimination of radioactivity was rapid, primarily via the urine and faeces. Three days after dosing with [^{14}C-chlorophenyl]fenvalerate, 31.6% and 55.5% had been eliminated in the urine and faeces, respectively. Total recovery of radioactivity was higher in animals dosed with [^{14}C-chlorophenyl]fenvalerate than in those dosed with [^{14}C-phenoxybenzyl]fenvalerate. The half-lives were 1 day and 0.7 day for radioactivity administered as [^{14}C-chlorophenyl]- and [^{14}C-phenoxybenzyl]fenvalerate, respectively. Maximum levels of radioactivity in the blood (approximately 1 µg/ml) were obtained 2 hours after dosing. Radioactivity rapidly decreased in more than two phases. The blood levels 80 hours after dosing were less than 0.05 and 0.05–0.1 µg/ml in animals dosed with [^{14}C-chlorophenyl]- and [^{14}C-phenoxybenzyl]fenvalerate, respectively. The level of the parent compound was below the limit of detection (0.01 mg/l) 48 hours after dosing (Kaneko et al., 1984).

1.2 Biotransformation

Investigation of the metabolites of [2*S*,α*RS*]-fenvalerate and fenvalerate produced by male Sprague-Dawley rats was carried out by one-dimensional TLC using two solvent systems with detection by autoradiography. Metabolites were either identified using co-chromatography with authentic standards or extracted from the gel and identified using infrared spectroscopy, mass spectrometry or nuclear magnetic resonance (NMR).

Fenvalerate undergoes several major metabolic reactions: cleavage of the ester linkage, hydroxylation in the acid and alcohol moieties and conversion of the cyano (CN) group to thiocyanate (SCN) and carbon dioxide. The resulting metabolite acids and phenols are subsequently conjugated with glucuronic acid, sulfuric acid or amino acids. More than 20 metabolites were identified. The major radioactive products in the faeces were unmetabolized fenvalerate and two ester metabolites (2′-OH-Fen and 4′-OH-Fen). No unmetabolized fenvalerate was found in the urine. The major urinary metabolites were CPIA, 3-phenoxybenzoic acid (PBacid), SCN^- and/or products of further oxidative and conjugation reactions. As it was determined that the excretion, tissue residues and metabolite excretion patterns between fenvalerate and esfenvalerate were similar, it was concluded that both compounds would be metabolized in a similar way. The significant metabolic reactions were oxidation at the 2- and 4-positions of the acid and at the 2′- and 4′-positions of the alcohol moiety, cleavage of the ester linkage and conversion of the cyano group to SCN^- and carbon dioxide (Ohkawa et al., 1979).

In a study using male and female Sprague-Dawley rats and ddY mice dosed orally with ^{14}C-labelled acid, alcohol and cyano moieties of fenvalerate and the [2*S*,α*S*]-isomer (esfenvalerate), the

metabolites found were essentially the same in both species, with no differences between the isomers, doses or sexes. Differences between the species were observed in the nature and amounts of metabolites derived from the alcohol moiety. These were as follows:

- 4′-hydroxylation was greater in rats than in mice;
- PBacid was taurine conjugated in mice, but not in rats;
- sulfate conjugation of 3-(4′-hydroxyphenoxy)benzoic acid (4′-OH-PBacid) was greater in rats than in mice;
- conversion of the cyano group to SCN^- was greater in mice than in rats.

There was little difference between the species in the elimination of acid- and alcohol-labelled fenvalerate and esfenvalerate. Mice eliminated the cyano-labelled compounds more quickly and more completely than rats. Higher doses of acid- and alcohol-labelled compounds were excreted at a slower rate than lower doses, and the cyano labels were more slowly excreted than the acid and alcohol labels (Kaneko, Ohkawa & Miyamoto, 1981).

In a 28-day feeding study in mice, the major metabolites in the liver and kidney of animals fed [^{14}C-chlorophenyl]esfenvalerate were CPIA and the hydroxylated derivative of CPIA. These disappeared after the mice were fed untreated diets. These metabolites were also found in mice fed [^{14}C-chlorophenyl]fenvalerate. In addition, CPIA–cholesterol ester was found in mice fed [^{14}C-chlorophenyl]fenvalerate, but not in mice fed [^{14}C-chlorophenyl]esfenvalerate. This metabolite was also present at the end of the 28 days on untreated diets and accounted for the majority of the radioactivity present at that time (Isobe et al., 1985).

In a separate study, rats fed 20 ppm fenvalerate in the diet for 28 days were sacrificed, and residues in adipose tissue were examined. Based upon chromatographic and mass spectral analysis, the residues in fat were characterized as unchanged fenvalerate containing both diastereoisomers (Boyer, 1977).

In a placental transfer study with rats, metabolites were tentatively identified by co-chromatography with standards, using TLC and five solvent systems, visualized by autoradiography and ultraviolet (UV) fluorescence and quantified by LSC. The major metabolites, CPIA and 3-OH-CPIA in its free and lactone forms, and the parent compounds were found in the maternal blood and placenta and fetuses. A trace of the CPIA–cholesterol ester [cholesteryl (2*R*)-2-(4-chlorophenyl)isovalerate], derived from [^{14}C-chlorophenyl]fenvalerate, was found in the maternal blood and placenta, but not the fetuses. Consecutive treatment resulted in slightly higher 3-OH-CPIA levels in rats dosed with esfenvalerate than with fenvalerate and slightly higher CPIA levels after dosing with fenvalerate than after dosing with esfenvalerate (Shiba et al., 1985).

After administration of a single oral dose or five consecutive oral doses of [^{14}C]esfenvalerate or [^{14}C]fenvalerate labelled in the acid moiety to 13-day pregnant rats at a rate of 2.5 or 10 mg/kg bw per day, respectively, major ^{14}C compounds in the fetus, maternal blood and placenta were the parent compounds, CPIA and CPIA-hydroxylated derivatives, and there was no qualitative difference in metabolic fates between the two compounds, except that a trace amount of CPIA–cholesterol ester was detected in the maternal blood and placenta only with fenvalerate. CPIA–cholesterol ester did not seem to transfer from the maternal blood to the fetus (Shiba et al., 1990).

In a study with dogs given a single oral dose of ^{14}C-chlorophenyl- and ^{14}C-phenoxybenzyl-labelled fenvalerate, excreta were analysed for metabolites that were tentatively identified and quantified by TLC co-chromatography with standards, using six solvent systems, either with or

without enzymatic hydrolysis. Visualization was by UV fluorescence and autoradiography. The major metabolites 0–1 day after dosing are shown in Table 3.

Table 3. Excreted metabolites in dogs following a single oral dose of [^{14}C]fenvalerate (1.7 mg/kg bw)

		% of administered radioactivity			
		[^{14}C-Chlorophenyl]		[^{14}C-Phenoxybenzyl]	
		Urine	Faeces	Urine	Faeces
Fenvalerate		0.2	8.8	—	3.7
4′-OH-Fen		—	1.8	—	2.3
PBalc		—	—	—	1.3
4′-OH-PBalc	Free	—	—	—	3.3
	Glucuronide conjugate	—	—	0.6	—
PBacid	Free	—	—	3.1	1.4
	Glucuronide conjugate	—	—	—	0.4
	Glycine conjugate	—	—	8.4	
4′-OH-PBacid	Free	—	—	2.4	3.5
	Sulfate conjugate	—	—	5.8	1.1
	Glucuronide conjugate	—	—	3.8	—
CPIA	Free	2.2	3.2	—	—
	Glucuronide conjugate	27.3	—	—	—
3-OH-CPIA	Free	2.9	—	—	—
	Glucuronide conjugate	4.9	—	—	—
3-OH-CPIA-lactone		—	1.9	—	—
2,3-OH-CPIA		3.4	—	—	—

From Kaneko et al. (1984)
CPIA, 2-(4-chlorophenyl)isovaleric acid; 2,3-OH-CPIA, 2-(4-chlorophenyl)-2,3-dihydroxymethyl-butenoic acid; 3-OH-CPIA, 2-(4-chlorophenyl)-3-hydroxymethyl-butenoic acid; 3-OH-CPIA-lactone, 2-(4-chloro-phenyl)-3-methylbutane-4-olide; 4′-OH-Fen, α-cyano-3-(4′-hydroxyphenoxy)benzyl-2-(4-chlorophenyl) iso-valerate; 4′-OH-PBacid, 3-(4′-hydroxyphenoxy)benzoic acid; 4′-OH-PBalc, 3-(4′-hydroxyphenoxy)benzyl alcohol; PBacid, 3-phenoxybenzoic acid; PBalc, 3-phenoxybenzyl alcohol

Although total recovery of radioactivity was less in dogs than in rats and mice, the disappearance pattern and half-lives in dogs and rats were similar. The following species differences in the metabolism were noted:

- 2′-hydroxylation of the alcohol moiety did not occur in dogs;
- 3-phenoxybenzyl alcohol (PBalc) and 3-(4′-hydroxyphenoxy)benzyl alcohol (4′-OH-PBalc) were detected in dogs only;
- 3-phenoxybenzoylglycine (glycine conjugate of PBacid) was the predominant conjugate of the alcohol moiety in dogs, but a minor one in rats;
- glucuronides of the acid moiety and its hydroxy derivatives were greater in dogs (Kaneko et al., 1984).

The excreta of the rats in an earlier comparative study were analysed for metabolites by TLC using five solvent systems, autoradiography and LSC, sometimes after enzyme treatment. The metabolites were tentatively identified by co-chromatography with unlabelled standards, which were visualized by UV fluorescence. There was no difference in metabolite excretion between the sexes or compounds. In faeces, 44.5–59.9% of the administered radioactivity from the ^{14}C-chlorophenyl- or ^{14}C-phenoxybenzyl-labelled materials was excreted as the parent compound, whereas 2.9–6.5% of the ^{14}C-chlorophenyl-labelled materials was excreted as the 2′-OH parent compound (2′-OH-Fen) and 4′-OH parent compound (4′-OH-Fen). The same amount of ^{14}C-phenoxybenzyl-labelled material was excreted as 4′-OH-Fen.

Other faecal metabolites were CPIA, its 3- and 2,3-hydroxy derivatives (3-OH-CPIA and 2,3-OH-CPIA) and 2-(4-chlorophenyl)-*cis*-2-butenedioic acid (Cl-BDacid) in their free forms from the acid moiety, and PBacid and 3-(4′-hydroxyphenoxy)benzoic acid (4′-OH-PBacid) in their free forms from the alcohol moiety. The major urinary metabolites from the acid moiety were CPIA-glucuronide, 3-OH-CPIA (free and lactone), 2,3-OH-CPIA–glucuronide and Cl-BDacid anhydride. The major urinary metabolite from the alcohol moiety was 4′-OH-PBacid sulfate (16.4–23.9% of the administered radioactivity). Other metabolites included PBacid (free and as glycine and glucuronide conjugates), 4′-OH-PBacid (free and as glucuronide) and 2′-OH-PBacid (free and as sulfate) (Kaneko et al., 1985).

The excreta of mice dosed with ^{14}C-acid- and ^{14}C-alcohol-labelled fenvalerate and esfenvalerate, as described above, were analysed for metabolites as above. There were no significant sex differences in metabolite excretion. The only difference between esfenvalerate and fenvalerate was that a trace amount of the cholesterol ester of CPIA was found in the faeces of mice treated with [^{14}C-chlorophenyl]fenvalerate. In faeces, 25.6–48.1% of the radioactivity from administration of ^{14}C-chlorophenyl- or ^{14}C-phenoxybenzyl-labelled materials was excreted as the parent, 1.4–4.4% was excreted as 4′-OH-Fen and 0.0–1.1% was excreted as 2′-OH-Fen. The other faecal metabolites were as found in the rats. The acid-labelled urinary metabolites mainly consisted of CPIA (free and glucuronide) and 2,3-OH-CPIA–glucuronide. The major alcohol-labelled urinary metabolites were the taurine conjugate of PBacid (7.9–17.3% of the administered radioactivity) and the free, sulfate and glucuronide forms of 4′-OH-PBacid (6.9–9.9% of the administered radioactivity). The minor metabolites were predominantly the same as those found in the rats (Kaneko et al., 1985).

The identification of the [2*R*,α*S*]-isomer of fenvalerate as the only source of CPIA–cholesterol ester was established. [^{14}C-chlorophenyl]Fenvalerate was administered to groups of two male Sprague-Dawley rats and four male ddY mice as a single oral dose of 2.5 mg/kg bw as the [2*S*,α*S*]-, [2*S*,α*R*]-, [2*R*,α*S*]- or [2*R*,α*R*]-isomers of fenvalerate. Excreta were collected for 6 days, and the radioactivity in the tissues and excreta was analysed by LSC. The residues of [2*S*,α*S*]-, [2*S*,α*R*]- and [2*R*,α*R*]-isomers were very low (100 µg equivalents per kilogram or less) in all tissues except fat (310–760 µg equivalents per kilogram) in both species. However, administration of the [2*R*,α*S*]-isomer resulted in higher residues in all tissues, particularly the adrenals, liver, mesenteric lymph nodes and spleen, although residues in fat were similar to those found as a result of administration of the other isomers. Thus, this lipophilic tissue residue does not occur to any appreciable extent in rats or mice dosed with esfenvalerate (Kaneko, Matsuo & Miyamoto, 1986).

To demonstrate this more clearly, groups of male ddY mice were fed diets containing [2*R*,α*S*]-, [2*S*,α*S*]- or [2*R*,α*R*]-isomer at 500 mg/kg bw for 1 or 2 weeks. Similar results were obtained, and further analysis by TLC showed that a lipophilic metabolite was predominant in all tissues of the mice treated with the [2*R*,α*S*]-isomer only. The liver of the [2*R*,α*R*]-treated mice contained trace amounts of the lipophilic metabolite. The lipophilic metabolite was identified by NMR or TLC co-chromatography, with authentic standards, using three different solvent systems, high-performance

liquid chromatography and mass spectrometry and was found to be the CPIA–cholesterol ester (Kaneko, Matsuo & Miyamoto, 1986).

An investigation of the mechanism of the formation of the CPIA–cholesterol ester from the [2*R*,α*S*]-isomer was conducted. Various tissues, including brain, kidney, liver, spleen, adrenals, intestines and lymph nodes, were excised from male Sprague-Dawley rats, ddY mice, Beagle dogs and Rhesus monkeys. The 9000 × *g* supernatant and pellet, microsomal fraction (100 000 × *g* pellet) and 100 000 × *g* supernatant were obtained by centrifugation. Plasma was obtained from blood by centrifugation. Incubation of each of the ^{14}C-labelled isomers of fenvalerate with the 9000 × *g* supernatants revealed that the [2*S*,α*S*]-, [2*S*,α*R*]- and [2*R*,α*R*]-isomers produced the hydrolysis product, CPIA, only, whereas the [2*R*,α*S*]-isomer resulted in the formation of CPIA and the CPIA–cholesterol ester. The production of CPIA depended on the tissue. In mice, it was most rapidly formed from the [2*R*,α*S*]-isomer in the kidney, brain and spleen, from the [2*R*,α*S*]- and [2*R*,α*R*]-isomers in the liver and from the [2*S*,α*R*]- and [2*R*,α*R*]-isomers in plasma.

CPIA–cholesterol ester was formed only from the [2*R*,α*S*]-isomer in all tissues and species examined, apart from the mouse kidney, which produced only a trace amount from the [2*R*,α*R*]-isomer. There were some species differences in the enzymatic activities of various tissues, but in general, the greatest activities were obtained from mouse tissues. Mouse kidney, brain and spleen produced the most CPIA–cholesterol ester. Free CPIA was not a substrate for the formation of CPIA–cholesterol ester. The activity was destroyed by heat, inhibited by tetraethylpyrophosphate (TEPP) and not affected by *p*-chloromercuribenzoic acid. Incubation of the [2*R*,α*S*]-isomer with subcellular fractions of mouse brain, liver, kidney and spleen showed that most of the CPIA–cholesterol ester–forming activity was located in the microsomes (Miyamoto, Kaneko & Takamatsu, 1986).

The role of three known routes of cholesterol ester synthesis in the formation of CPIA–cholesterol ester was investigated. Mouse liver microsomes were incubated with oleic acid or CPIA with and without adenosine triphosphate, coenzyme A and TEPP. Although the CPIA–cholesterol ester was formed, this activity was inhibited by TEPP, whereas TEPP had no effect on the oleic acid–cholesterol ester formation. This indicated that acyl coenzyme A–cholesterol acyl transferase was not involved in the formation of the CPIA–cholesterol ester.

Mouse plasma and kidney microsomes were incubated with lecithin or [2*R*,α*S*]-fenvalerate. Lecithin–cholesterol ester was obtained from plasma but not kidney microsomes, and CPIA–cholesterol ester was obtained from kidney microsomes but not plasma. This showed that lecithin–cholesterol acyl transferase was not involved in the formation of the CPIA–cholesterol ester. Incubation of oleic acid, [2*R*,α*S*]-fenvalerate or CPIA with intestinal mucosal microsomes or intestinal mucosal 100 000 × *g* supernatant and cholesterol resulted in a cholesterol ester from oleic acid only. This showed that cholesterol esterase was not involved.

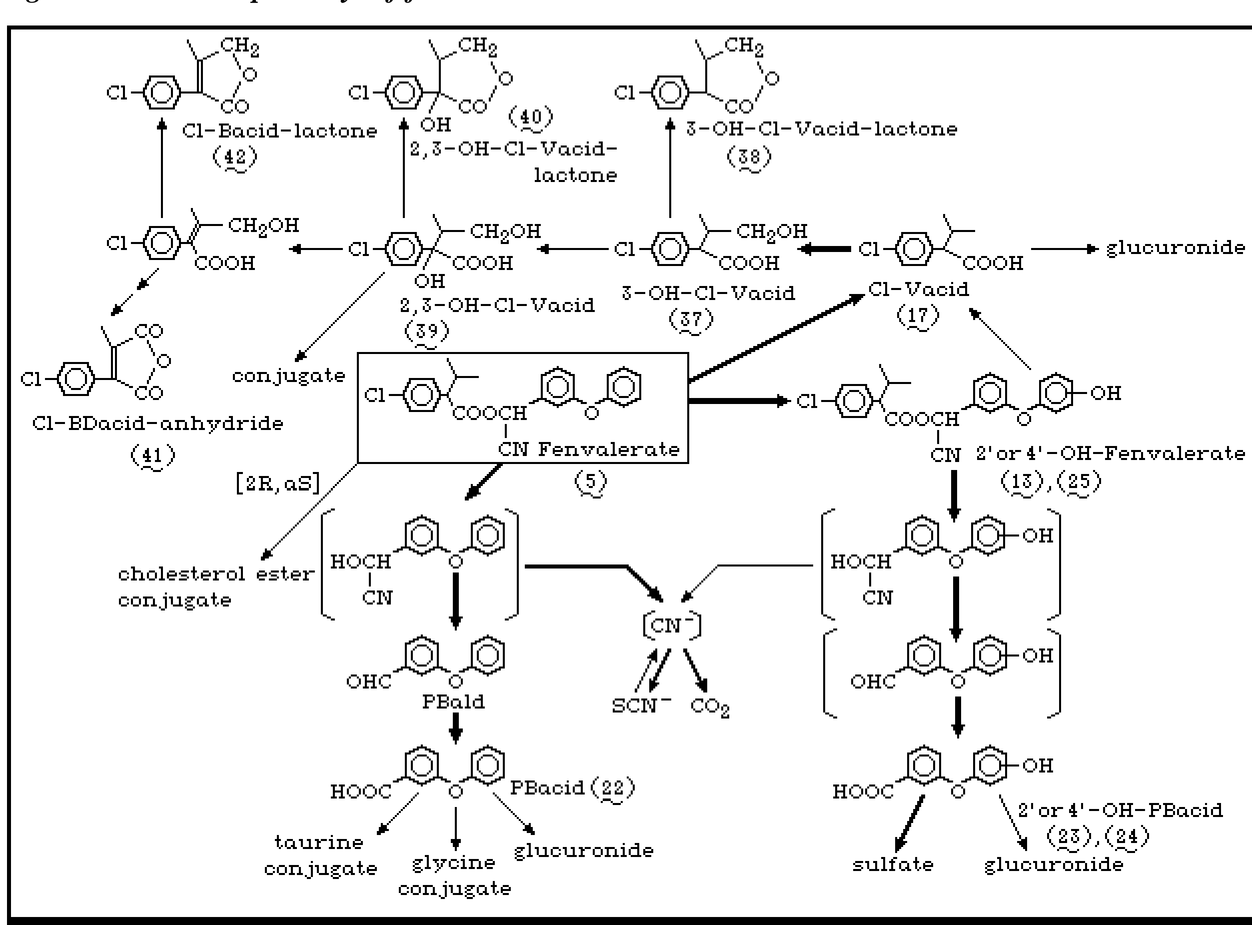
In order to determine the enzyme responsible for the formation of the CPIA–cholesterol ester, mouse kidney and liver microsomes were incubated with [2*R*,α*S*]-fenvalerate only or [2*R*,α*S*]-fenvalerate and methanol, ethanol, *n*-propanol or *n*-butanol. It was found that in the presence of the straight-chain alcohols, the amount of CPIA–cholesterol ester was reduced and the amount of CPIA–alkyl ester was increased with increasing chain length. In addition, incubation of mouse kidney microsomes with [2*R*,α*S*]-isomer and cholesterol or cholesterol oleate resulted in the production of CPIA–cholesterol ester from cholesterol only. Therefore, the CPIA–cholesterol ester is produced from free cholesterol, and alkyl alcohols also act as “receptors” for CPIA. It was inferred that the formation of the CPIA–cholesterol ester from [2*R*,α*S*]-fenvalerate was a transesterification reaction mediated by microsomal carboxylesterase(s). When mouse kidney microsomes were solubilized with digitonin, they retained the hydrolysis activity but did not produce the CPIA–cholesterol ester. Addition of liposomes to the solubilized enzyme restored the cholesterol ester–forming activity. These findings

suggest that, in microsomes, the carboxylesterase(s) and cholesterol are spatially arranged so that cholesterolysis of the intermediary CPIA–enzyme complex is possible, resulting in the formation of CPIA–cholesterol ester (Miyamoto, Kaneko & Takamatsu, 1986; Kaneko et al., 1988).

Photolysis of fenvalerate in solvents by artificial light and as a thin film on glass or cotton by sunlight yields products resulting from ester cleavage. The major pathway in solution was identified as a photo-induced decarboxylation yielding an unusual product, not known to occur in mammals (Holmstead, Fullmer & Ruzo, 1978).

The metabolic pathways of fenvalerate in mammals are illustrated in Figure 3.

Figure 3. Metabolic pathways of fenvalerate in mammals



Metabolic pathways of fenvalerate in mammals.

2. Toxicological studies

2.1 Acute toxicity

The results of acute toxicity studies with fenvalerate are summarized in Table 4. All the studies were in compliance with European Union guidelines of the day and either in compliance with GLP or, where pre-1993, conducted and reported to an adequate standard.

Table 4. Acute toxicity of fenvalerate

Species	Sex	Route	Vehicle	Result (LD_{50} or LC_{50})	Reference
Mouse	M, F	Oral	DMSO	M: 200–300 mg/kg bw F: 100–200 mg/kg bw	Walker, Hend & Linnett (1975)
Mouse	NS	Oral	PEG/water	1202 mg/kg bw	Summitt & Albert (1977b)
Rat	NS	Oral	DMSO	451 mg/kg bw	Walker, Hend & Linnett (1975)
Rat	NS	Oral	PEG/water	> 3200 mg/kg bw	Summitt & Albert (1977a)
Chinese hamster	M, F	Oral	DMSO	M: 98 mg/kg bw F: 82 mg/kg bw	Walker, Hend & Linnett (1975)
Syrian hamster	NS	Oral	PEG/water	~760 mg/kg bw	Hart (1976a)
Dog	NS	Oral	PEG/water or corn oil	100–1000 mg/kg bw emetic	Hart (1976b)
Hen	NS	Oral	NS	> 1500 mg/kg bw	Milner & Butterworth (1977)
Rat	NS	Dermal	NS	5000 mg/kg bw	Okuno, Kadota & Miyamoto (1976)
Rabbit	NS	Dermal	Undiluted	1000–3200 mg/kg bw	Hine (1975)
Rat	M, F	Inhalation	Water	> 101 mg/m^3 (3 h)	Kohda, Kadota & Miyamoto (1976b)
Mouse	M, F	Inhalation	Water	> 101 mg/m^3 (3 h)	Kohda, Kadota & Miyamoto (1976b)
Mouse	M, F	Intraperitoneal	Corn oil	58–89 mg/kg bw	Kohda et al. (1979)
Mouse	NS	Intravenous	Glyceroformol	65 mg/kg bw	Albert & Summitt (1976)

DMSO, dimethyl sulfoxide; F, female; LC_{50}, median lethal concentration; LD_{50}, median lethal dose; M, male, NS, not specified; PEG, polyethylene glycol

Within 4 hours of dosing, all animals receiving acutely toxic levels were restless and developed tremors, piloerection, occasional diarrhoea and an abnormal gait. Following oral administration, animals recovered rapidly from acute clinical signs of poisoning and were asymptomatic within 3–4 days. Immediately after exposure, rats showed an abnormal gait, which is typical of pyrethroid intoxication. The animals walked with hindquarters held up and the hind legs more widely spaced than normal (splayed). Histological examination of the sciatic nerve and posterior tibial nerve, after poisoning and for 9 days over the course of recovery, showed axonal breaks, swelling and vacuolation accompanied by vacuolation and phagocytosis of myelin. The degree to which myelin was disrupted was dose dependent and was closely associated with the acute signs of toxicity (Butterworth & Carter, 1976).

No data are available on skin and eye irritation or skin sensitization. However, data are available on esfenvalerate, which is not irritating to the skin and minimally irritating to the unwashed eyes of rabbits. It was a skin sensitizer in guinea-pigs using the Magnusson and Kligman

maximization test, but was not a sensitizer using the Buehler test method (Annex 1, references 95 and 97).

2.2 *Short-term studies of toxicity*

(a) Oral administration

Mice

In a 10-week published dose range–finding study, groups of 4–8 male and female C57B1/6 mice received fenvalerate (purity 99%) via gavage at 0, 20, 40, 80 or 160 mg/kg bw per day in arachis oil, 5 days/week for 10 weeks. All animals were necropsied and examined histopathologically.

At 160 mg/kg bw per day, four male and four female mice died the day after the treatment, and three male and three female mice died before the end of the experiment, probably due to gavage error at 80 mg/kg bw per day. All animals at 80 and 160 mg/kg bw per day were agitated for several hours after the treatment. No dose-related gross pathology was observed in any group. Treatment-related liver, spleen and lymph node microgranulomas were observed in mice surviving the 10-week treatment schedule. No such lesions were observed in control mice (Cabral & Galendo, 1990).

Rats

In a no-effect level determination study for neurotoxicity, male and female Sprague-Dawley rats were exposed to fenvalerate (purity 97.7%) in diets mixed with corn oil for 7 or 9 days for neuropathological examination. In the first study, 8 male and 8 female rats and 24 male and 24 female rats were treated at 0 or 3000 ppm (equivalent to 0 and 300 mg/kg bw per day), respectively. In another study, 10 rats of each sex were administered fenvalerate at 0, 500 or 1500 ppm (equivalent to 0, 50 and 150 mg/kg bw per day, respectively). During the whole feeding period, clinical signs and mortalities were checked daily. Feed consumption and body weight were recorded twice during the feeding period. The surviving rats were sacrificed on days 7–9 for neuropathological examinations.

Rats administered the highest dose of fenvalerate developed severe toxic symptoms, but the rats of other groups showed no toxic symptoms, except for those in the 1500 ppm group, which had slight hypersensitivity. Clinical signs of intoxication appeared within 24–48 hours, characterized by hypersensitivity, erratic jumping, tremor and hindlimb ataxia. Eight male and six female rats in the 3000 ppm group died within 8 days after treatment. Body weight and feed consumption were reduced at 1500 and 3000 ppm. Sciatic nerves of rats at the highest dose showed swelling and, in some rats, disintegration of the axons. Sex differences in these lesions were not found. The sciatic nerve changes were minute and sporadic at lower doses and occasionally found in control rats.

Thus, the histopathological no-effect level for fenvalerate was 1500 ppm (equivalent to 150 mg/kg bw per day), although the rats in this group showed slight symptoms. Therefore, the neurotoxic effects are considered to be found only in rats receiving lethal or near-lethal doses (Miyamoto, Okuno & Kadota, 1977).

Groups of six male and six female Carworth Farm E rats were fed diets containing 0 or 2000 ppm (equivalent to 0 and 200 mg/kg bw per day) of fenvalerate (WL 43775, purity 98.0%). A randomized block design was used. Daily observations were made on the general health and behaviour of each animal. After 8 days of feeding in the males and 10 days in the females, both treated and control animals were killed for pathological examination. Following gross pathological examination, sections of sciatic nerve were stained by the cresyl violet–luxol fast blue method for myelin and by the Glees and Marsland method for axons and then examined histologically.

In all male and female rats exposed to 2000 ppm fenvalerate, there were typical signs of ataxia, tremors and some hypersensitivity to noise indistinguishable from pyrethroid intoxication. Pathological examination of the 11 sciatic nerves of exposed rats (one sciatic nerve was lost) did not reveal neuropathological changes (Stevenson, Hend & Butterworth, 1976).

In a 90-day toxicity study, groups of Carworth Farm E rats (12 males and 12 females per group) were fed fenvalerate (98%) in the diet at a dose level of 0, 125, 500, 1000 or 2000 ppm (equivalent to 0, 12.5, 50, 100 and 200 mg/kg bw per day, respectively) for 90 days. Mortality (11/12 males and 9/12 females) was observed at 2000 ppm. Body weight gain and feed consumption were decreased at 1000 and 2000 ppm. With the exception of an increase in blood urea nitrogen concentration at 1000 and 2000 ppm, haematological and clinical chemistry examinations at the conclusion of the study were normal. Gross and microscopic examinations of tissues and organs were performed at the conclusion of the study. Increases in liver to body weight and kidney to body weight ratios were observed at 500 ppm and above. These gross changes were not accompanied by observable microscopic changes on histopathological examination. Sciatic nerves, examined at the conclusion of the study, showed no myelin or axonal degeneration.

The NOAEL was 125 ppm (equivalent to 12.5 mg/kg bw per day), based on increases in relative liver and kidney weights seen at 500 ppm (equivalent to 50 mg/kg bw per day) (Hend & Butterworth, 1975).

Hamsters

In a short-term dietary toxicity study, 12 male and 12 female Golden Syrian hamsters were fed diet containing fenvalerate (SD-43775, technical grade, purity was not specified) at 0, 1, 10, 100 or 1000 ppm (equivalent to 0, 0.1, 1, 10 and 100 mg/kg bw per day, respectively) for 28 days.

No dose-related effects on body weight or feed consumption were noted. At postmortem examination, no gross pathology was apparent, but decreases in spleen weight (males at 1000 ppm, females at all dose levels) and adrenal weights (males at 100 and 1000 ppm, females at all but the lowest dose level) and increases in liver weight (high-dose females only) were noted (Hart, 1975a).

In a dose range–finding study, groups of 4–8 male and female Syrian Golden hamsters received fenvalerate (purity 99%) via gavage at 0, 20, 40, 80 or 160 mg/kg bw per day in arachis oil, 5 days/week for 10 weeks. All animals were necropsied and examined histopathologically.

All hamsters survived to the end of the study. Slight hepatocyte hypertrophy was observed in those hamsters receiving fenvalerate at 80 and 160 mg/kg bw per day. No microgranulomatous changes were observed in any organs (Cabral & Galendo, 1990).

Dogs

In a short-term toxicity study, groups of young adult Beagle dogs (four males and four females per group) were fed fenvalerate (SD 43775, purity 98.0%) in the diet at a dose level of 0, 0.05, 0.25, 1.25 or 12.5 mg/kg bw per day for 90 days.

There were no abnormalities over the course of the study. Body weight gain, feed consumption and behaviour were normal. Results of clinical laboratory examinations performed 3 times during the course of the study showed no effects of fenvalerate administration in the diet. At the conclusion of the study, data from gross and microscopic examinations of a variety of tissues and organs substantiated the clinical data, again showing no effects of dietary fenvalerate administration. Daily administration at a dose of 12.5 mg/kg bw per day for a period of 90 days produced no detectable evidence of toxicological effects.

The NOAEL was 12.5 mg/kg bw per day, the highest dose tested (Hart, 1975b).

In a 6-month toxicity study, male and female Beagle dogs (six of each sex per group) were fed diets containing fenvalerate (purity 91%) at 0, 250, 500 or 1000 ppm (equivalent to 0, 18.8, 37.5 and 75.0 mg/kg bw per day, respectively).

Prominent clinical signs related to treatment were emesis, head shaking, biting of the extremities and tremors. The mean body weights of female dogs fed fenvalerate at 1000 ppm were significantly lower than those of controls. Red blood cell counts and haematocrit and haemoglobin values in both male and female dogs fed the highest dose were significantly lower than those of controls. Serum cholesterol and alkaline phosphatase levels were also increased, mostly in the group fed 1000 ppm. Hepatic multifocal microgranulomas observed during microscopic examination increased in incidence and severity in a dose-dependent manner and were considered to be related to treatment. Histiocytic cell infiltrates in the mesenteric lymph nodes of some female dogs fed 500 or 1000 ppm and of male dogs fed 1000 ppm were the only other treatment-related effects observed microscopically.

The NOAEL was 250 ppm (equivalent to 18.8 mg/kg bw per day), based on microscopic findings in the mesenteric lymph nodes and histiocytic cell infiltrates in females seen at 500 ppm (equivalent to 37.5 mg/kg bw per day) (Piccirillo, 1981; Parker et al., 1984).

(b) Dermal application

Rats

In a dermal neurotoxicity study, male Sprague-Dawley rats were exposed to fenvalerate (purity 97.7%) via dermal application. Four rats were treated dermally with a single application of fenvalerate and NRDC-149 (cypermethrin) at 5000 mg/kg bw. Another group of 10 males was treated dermally with fenvalerate and NRDC-149 at 2500 and 5000 mg/kg bw per day for five consecutive repeated applications. The single-application rats were killed on day 5, and the repeated-exposure groups were killed on day 7.

Toxic symptoms, such as hypersensitivity, tremor and hindlimb ataxia, were observed in all rats of each treated group, except that hindlimb ataxia was not seen with a single application of 5000 mg/kg bw of fenvalerate. Some animals in each repeated-dose group except NRDC-149 at 2500 mg/kg bw died during the observation period. Lesions such as swelling and/or disintegration of axon in sciatic nerves of all rats treated with fenvalerate and NRDC-149 were found histopathologically. The changes were related to total dosage of the test compounds. The degree of lesion in fenvalerate-treated groups was less than that in NRDC-149-treated groups. The above results suggest that the dermal application of lethal or near-lethal doses of fenvalerate and NRDC-149 caused neurotoxic effects in male rats (Okuno & Kohda, 1976).

Rabbits

In a 21-day dermal toxicity study, groups of rabbits (7–8 males per group) were administered fenvalerate dermally at a dose level of 0, 100 or 400 mg/kg bw per day for 6 hours/day (14 exposures were performed over a 22-day period).

Mortality was observed at the high dose level, accompanied by severe weight loss, clinical signs of poisoning and gross dermal effects. Rabbits tolerated the 100 mg/kg bw per day dose with minor local dermatological effects (Hine, 1975).

In a 21-day dermal toxicity study, fenvalerate (DPX-Y4306-90, purity 95.4%) was applied to the shaved skin of five New Zealand White rabbits of each sex per dose at a dose level of 0, 100, 300, or 1000 mg/kg bw per day, 6 hours/day, during a 21-day (male) or 22-day (female) period consecutively under occlusive dressing. A control group (five of each sex) was similarly treated with deionized water. After an exposure period of approximately 6 hours, the bandages were removed and the test substance was removed by washing. The test substance was not soluble or miscible in water; therefore, the test site was first washed with dilute (50–70%) acetone, followed by an Ivory® soap and water solution to remove excess test substance. The test site of each rabbit was then gently rinsed with lukewarm water, and the skin was patted dry. Control animals received the same washing technique as treated animals. At this time, the animals were observed for dermal irritation and clinical signs of toxicity. Dermal irritation was scored according to the Draize scale. All surviving male and female

rabbits were killed by design for gross and microscopic pathological examinations following blood collection on test days 22 and 23, respectively.

There were no compound-related effects on mortality, clinical signs, body weight, feed consumption, feed efficiency, haematology, clinical chemistry, organ weights, or gross and histological pathology. There were treatment-related increased incidences of dermal irritation, which included the following: moderate erythema in 2/8, 2/10, 4/8 and 4/10 animals; mild oedema in 0/8, 1/10, 3/8 and 3/10 animals; superficial necrosis in 0/8, 0/10, 3/8 and 4/10 animals; and scar tissue in 0/8, 0/10, 3/8 and 2/10 animals in the control, low-dose, mid-dose and high-dose groups, respectively.

The NOAEL for systemic toxicity was 1000 mg/kg bw per day, the highest dose tested (MacKenzie, 1992).

(c) Exposure by inhalation

In a 4-week inhalation toxicity study, groups of Sprague-Dawley rats and ICR mice (10 males and 10 females of each species per group) were administered fenvalerate by inhalation exposure 4 hours daily for 4 weeks at a concentration of 0 or 20 mg/m^3. Histopathological examinations were performed on various tissues/organs.

No treatment-related findings were observed in a comparison between treated groups of rats and mice and corresponding controls (Ito, 1976b).

In a second 4-week inhalation toxicity study, groups of Sprague-Dawley rats and ICR mice (10 males and 10 females of each species per group) were administered fenvalerate by inhalation exposure 3 hours daily for 4 weeks at a concentration of 0, 2, 7 or 20 mg/m^3. Animals were exposed to a small, fully respirable particulate (1–2 µm) during the course of the study.

Mortality was not noted over the course of the study, but animals at the high dose level showed acute toxic signs of poisoning. No treatment-related effects on body weight, haematology or clinical chemistry parameters were observed. Gross and microscopic examinations of tissues and organs at the conclusion of the study showed no changes that were related to the administration of fenvalerate. The sciatic nerve was not examined in this study (Kohda et al., 1976c).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a chronic toxicity and carcinogenicity study, groups of ddY mice (35–47 of each sex per group) were administered fenvalerate in the diet for 78 weeks at a dose level of 0, 100, 300, 1000 or 3000 ppm (equivalent to 0, 5, 15, 50 and 150 mg/kg bw per day, respectively). Body weights were recorded periodically. At 3 months and terminal sacrifice, haematological and biochemical changes were determined, and a gross macroscopic examination of tissues and organs was carried out.

In the early stages of the study, mortality was evident at 1000 and 3000 ppm. Body weight was depressed at 3000 ppm throughout the study and at 1000 ppm during the first 3 months. Hypersensitivity, excitability and other behavioural changes were observed in the 1st month of feeding at 1000 and 3000 ppm. A variety of haematological parameters were affected during the first 3 months of the study, predominantly at the high dose level, but no haematological changes were observed at the end of the study. Several biochemical changes were observed, including a decrease in alkaline phosphatase, an increase in blood urea nitrogen, an increase in leucine aminopeptidase (LAP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activities and a decrease in cholesterol and glucose occurring at 300 (sporadically), 1000 and 3000 ppm, with several of these parameters being affected at 100 ppm (e.g. glucose decrease in females). Biochemical changes noted at the 3-month sacrifice interval were not seen at the conclusion of the study (increased blood urea nitrogen, decreased glucose, etc.). There were gross changes in several organ weights and in organ to body weight ratios, predominantly in the liver in both male and

female mice exposed to 3000 ppm. Other organ weight changes were also observed in the kidney, heart, spleen, pituitary and ovary at the highest dose level. Microscopic examination suggested changes in the liver, mesenteric lymph nodes and kidney at the two highest dose levels. Multiple small necrotic foci in the liver and changes in the epithelial cells of the proximal convoluted tubules were noted, apparently related to the presence of fenvalerate in the diet. There were no indications in this study of tumorigenicity or carcinogenicity as a result of fenvalerate administration.

The NOAEL was 300 ppm (equivalent to 15 mg/kg bw per day), based on reduced body weights, clinical signs of hyperactivity, mortality and clinical chemistry changes seen at 1000 ppm (equivalent to 50 mg/kg bw per day). The NOAEL for carcinogenicity in mice was 3000 ppm (equivalent to 150 mg/kg bw per day), the highest dose tested (Suzuki, Kadota & Miyamoto, 1976; Ito, 1976a, 1978; Suzuki et al., 1977b).

In a second carcinogenicity study, groups of 50 male and 50 female B6C3F1 mice were fed fenvalerate (SD-43775, purity 98%) at a dietary concentration of 0, 10, 50, 250 or 1250 ppm (equivalent to 0, 1.5, 7.5, 37.5 and 187.5 mg/kg bw per day) for 2 years. Two groups of control mice, 50 of each sex per group, received basal diet only.

Mortality was significantly increased in male and female mice in the 1250 ppm treatment group. Mortality ranged from 19% to 56% and was largely unaffected by treatment. Mortality in the 1250 ppm group was 56% for males and 54% for females, these proportions being significantly greater than in the controls, but mortality also reached 54% for males in the 10 ppm group (Table 5). There were no compound-related signs of toxicity.

Table 5. Mortality in a 2-year carcinogenicity study in mice

Dietary concentration (ppm)	Males (dead or moribund)	%	Females (dead or moribund)	%
0	29/97	29.9	39/98	39.8
10	26/48	54.2	10/49	20.4
50	12/50	24.0	15/50	30.0
250	16/48	33.3	9/48	18.8
1250	27/48	56.3	27/50	54.0
Totals	110/291	37.8	100/295	33.9

From Johnston (1979)

Mean body weights and body weight gains of males and females fed 1250 ppm were significantly lower throughout the study, and those of females fed 250 ppm were also slightly, but significantly, lower from week 60 onwards. There were no significant changes in feed consumption or haematological parameters. Clinical biochemical changes were found in both sexes fed 1250 ppm, at which the AST level was increased and serum albumin levels were decreased. The observation of palpable masses did not show any dose-related trend, but it is clear that males had consistently more masses than females. At necropsy, there were no treatment-related macroscopic observations. For some organs, mean organ weights and organ to body weight ratios were statistically significantly different from controls. However, these changes were not consistent in the treatment groups (i.e. not dose related). Histopathology revealed no increases in neoplastic lesions. There was an increased incidence of microgranulomatous changes due to the treatment. In female mice, this lesion was present in mesenteric lymph nodes, liver and spleen at dose levels of 250 and 1250 ppm. In male mice, 1250 ppm fenvalerate induced multifocal granulomata in mesenteric lymph nodes, other visceral and peripheral lymph nodes, liver and spleen. Less severe granulomatous lesions were present in mesenteric lymph nodes of male mice administered 50 or 250 ppm fenvalerate.

In conclusion, the NOAEL was 10 ppm (equivalent to 1.5 mg/kg bw per day), based on the incidence of microgranulomatous changes in the spleen, liver and lymphatic tissue of males fed 50 ppm fenvalerate (equivalent to 7.5 mg/kg bw per day) (Johnston, 1979; McCullough & Gellatly, 1979; Parker et al., 1983).

In a third carcinogenicity study, groups of 50 male and 50 female ddY mice were fed fenvalerate (S5602 technical, purity 91.4%) at a dietary concentration of 0, 10, 30, 100 or 300 ppm for 87 (females) or 91 (males) weeks. Achieved mean compound intakes were 0, 1.24, 3.48, 12.3 and 36.0 mg/kg bw per day in males and 0, 1.42, 4.29, 13.6 and 40.8 mg/kg bw per day in females at 0, 10, 30, 100 and 300 ppm, respectively. Additional groups of 10 male and 10 female ddY mice were fed the same concentrations and were sacrificed at 12 months. This study is supplementary to the previously described study by Suzuki et al. (1977b) and was intended to establish a no-effect level.

No treatment-related effects, including mortality, body weight, feed consumption, water intake, ophthalmological examination and urine analysis, were detected by clinical observations. Slight reductions of erythrocyte counts in the 100 and 300 ppm groups of males and slightly lower haemoglobin concentration in the 300 ppm female group were observed. Blood glucose concentration was slightly lower in females fed 100 and 300 ppm, and ALT activities were slightly increased in females fed 300 ppm. There were no treatment-related changes in macroscopic findings at necropsy. No treatment-related effects were detected in organ weights or organ to body weight ratios, except that a slightly higher adrenal to body weight ratio was obtained in the 300 ppm female group at month 12. Histopathological examination revealed a dose-related increase in histiocytic infiltration (liver and lymph nodes) and the incidence of granulomatous changes in lymph nodes and liver of both sexes fed 100 and 300 ppm and in spleen of both sexes fed 300 ppm. There was no evidence of carcinogenicity to ddY mice.

The NOAEL for fenvalerate in ddY mice was concluded to be 30 ppm (equal to 3.48 mg/kg bw per day in males and 4.29 mg/kg bw per day in females), based on the slight decrease in erythrocyte counts, increased histiocytic infiltration (liver and lymph nodes) and granulomatous changes in the liver and lymph nodes in animals fed 100 ppm (equal to 12.3 and 13.6 mg/kg bw per day in males and females, respectively) (Arai et al., 1981b,c).

In a published carcinogenicity study, inbred C57Bl/6 mice (50 of each sex per dose) were given fenvalerate (purity 99%) by gavage at 0, 40 or 80 mg/kg bw per day, 5 days/week, for 120 weeks. Arachis oil was used as the vehicle to administer the fenvalerate. Two groups of mice (50 of each sex) were included as controls, one untreated controls and the other vehicle-treated controls.

Survival was decreased, especially among females receiving the high dose (Table 6).

Exposure to fenvalerate resulted in a slight increase in the incidence of liver cell tumours over that in controls only in male mice receiving the high dose. The incidence of liver tumours in high-dose males was 21% compared with 13% and 8% for untreated and vehicle-treated controls, respectively. The increased incidence of liver tumours in male mice at the high dose was not significant by Fisher's exact test, but was significant by the trend test ($P < 0.05$). No significant difference in the incidence of other types of tumours was observed in treated groups when compared with controls. Fenvalerate-induced microgranulomas occurred concomitantly in the liver, spleen and lymph nodes of male and female mice, but their overall incidence did not increase with dose. Considering the two sexes together, the incidence of these lesions was 35% in male mice receiving the low dose and 36.5% in the group receiving the high dose.

In a separate experiment, groups of female SJL/ola mice were administered two different samples of fenvalerate (purity 92% and 99%) once per week for 12 weeks. In animals that received 92% pure compound, the latent period for induction of lymphomas was shortened and their incidence increased, when compared with the group receiving 99% pure fenvalerate and with controls.

Table 6. Survival in mice administered fenvalerate for 120 weeks

Treatment	No. of each sex	No. of survivors							% survival
		10 weeks	20 weeks	40 weeks	60 weeks	80 weeks	100 weeks	120 weeks	
Controls									
Untreated	50 F	49	48	47	45	42	32	20	40
	50 M	50	50	50	48	48	47	32	64
Vehicle-treated	50 F	49	49	49	48	47	44	22	44
	50 M	49	48	48	46	42	38	28	56
Fenvalerate									
40 mg/kg bw per day	50 F	50	50	48	45	44	37	25	50
	50 M	50	50	48	48	46	42	29	58
80 mg/kg bw per day	50 F	57	56	51	44	40	29	17	34
	50 M	58	58	55	54	51	43	29	58

From Cabral & Galendo (1990)
F, females; M, males

The study authors concluded that the results of this study do not provide evidence for carcinogenicity of fenvalerate in C57Bl/6 mice (Cabral & Galendo, 1990).

Rats

Groups of rats (15 male and 15 female Wistar rats per group) were fed fenvalerate at a concentration of 0, 50, 150, 500 or 1500 ppm (equivalent to 0, 5.0, 15.0, 50 and 150 mg/kg bw per day) for 15 months in the diet.

There was no mortality in the study that was attributable to fenvalerate. Growth, feed consumption and behavioural changes were significantly affected at the highest dose level. Hypersensitivity was observed at the early stages of the experiment, disappearing within 3 months. Clinical examinations, performed at either 1 year (urine analysis) or the conclusion of the study (haematology, blood biochemistry and gross and microscopic pathology), showed significant abnormal values in a variety of parameters at the 1500 ppm dose level. No ophthalmological effects were noted. Haemoglobin concentration was depressed in males at the highest dose level and in females at 150 ppm and above. Blood biochemistry was significantly altered at 1500 ppm with respect to several parameters (blood urea nitrogen in both males and females; protein and cholinesterase in females). Gross and microscopic examination of tissues and organs, including specific sections of sciatic nerve and trigeminal ganglia and nerve, showed no dose-related effects. Generalized inflammatory and degenerative changes were seen in both control and treated animals. Tumour incidences were low and not related to the presence of fenvalerate. There was no suggestion of a carcinogenic potential observed in the study (Suzuki et al., 1977a). This study was not considered to be adequate for a carcinogenicity assessment of fenvalerate in rats, as inadequate numbers of animals were used per dose group and the duration of treatment was less than 2 years.

In a carcinogenicity study, groups of Sprague-Dawley rats (93 males and 93 females per group, 183 of each sex were used as the controls, and an additional 22 of each sex were used as separate control and high-dose groups) were fed fenvalerate (purity 98%) in the diet at a dose level of 0, 1, 5, 25, 250 or 500 ppm (equivalent to 0, 0.05, 0.25, 1.25, 12.5 and 25 mg/kg bw per day). After 3 and 6 months (all groups) and 12 and 18 months (0, 1, 5, 25 and 250 ppm groups) of treatment, rats

(10 of each sex per group, except the control group, 20 of each sex) were sampled for haematology and blood and urine chemistry, after which they were killed and examined for histopathology.

There was no effect of the treatment with fenvalerate on mortality, clinical signs, incidence of palpable masses (includes all palpable masses and not necessarily tumours), feed consumption, urine analysis data, haematological parameters, coagulation, clinical chemistry, serum protein or organ weight compared with the untreated controls. Some values of these parameters on some occasions were significantly different from the control values, but these alterations were not consistent and therefore are not considered treatment related. Body weight gain in females fed 500 ppm was slightly decreased during most of the treatment period (6 months). The 500 ppm group and a separate control group were sacrificed at 26 weeks, whereas the other animals were maintained for 2 years. There were no significant effects on feed consumption, growth or behaviour at 250 ppm. Haematology, clinical chemistry tests and urine analyses, performed at various time intervals over the course of the study on at least 10 animals of each sex at each dose level, showed no dose-related effects. At the conclusion of the study, organ weights and organ to body weight ratios were normal. Gross and microscopic examinations of tissues and organs did not differ significantly from controls. Benign and malignant lesions occurred at random throughout all groups examined at the end of the study and in those animals that died during the course of the study. There were no lesions attributable to fenvalerate. A specific pathological examination of the sciatic nerve of animals fed 250 ppm was performed. The sciatic nerve was not found to have been significantly affected by fenvalerate. This study was considered adequate for a carcinogenicity assessment of fenvalerate in rats.

The NOAEL was 250 ppm (equivalent to 12.5 mg/kg bw per day), based on a reduction in body weight gain in females fed 500 ppm (equivalent to 25 mg/kg bw per day) for a period of 6 months (Gordon & Weir, 1978).

A supplementary study was performed to complement the study by Gordon & Weir (1978) described above. A group of 50 male and 50 female Sprague-Dawley rats was fed fenvalerate (SD-43775 technical, 98% purity) at a dietary concentration of 1000 ppm (equivalent to 50 mg/kg bw per day) for 2 years. A concurrent group of 50 male and 50 female rats received basal diet only.

Six male rats of the treatment group developed transitory weakness of the hindlimbs during weeks 3 and 4 of the study. Five of the treated males developed skin lesions, typified by localized hair loss and oozing, slowly healing sores. Although the etiology of these lesions is unknown, these were judged unrelated to treatment with the test material. Mortality and feed consumption were not affected by compound administration. There was a decrease in body weight gain from week 16 in treated males and from week 44 in treated females to the end of the study. No compound-related changes were seen in the parameters of clinical chemistry, haematology or urine analysis. Organ weights of the treated group were similar to control values. As body weight gain was significantly decreased in the treated rats, the organ to body weight ratios were significantly greater ($P < 0.05$) in the treated males for brain, liver, heart, spleen and testes and in the treated females for brain, kidneys, liver, spleen and heart, when compared with control values.

There were no differences between the treated and control groups in the incidence of mammary tumours. Malignant neoplasms of mesenchymal origin (sarcomas) were observed in the subcutis and dermis of 5 of 51 male rats (2 terminal, 3 intercurrent) fed 1000 ppm compared with 0/50 in the controls. One of these lesions occurred in each of the cervical, hindlimb, thoracic, perianal and axillary regions. The characteristics of these lesions varied in different portions of the individual tumours, and there were differences among the neoplasms. They were, however, predominantly collagenous and generally contained frequent mitoses. Because the cells of origin could not be determined, they were simply designated as spindle cell sarcomas. The term spindle cell sarcoma encompasses a number of malignant mesenchymal neoplasms that are difficult and sometimes impossible to diagnose definitively and include fibrosarcoma, neurofibrosarcoma, malignant fibrous histiocytoma, liposarcoma and osteosarcoma. A spindle cell sarcoma also occurred in 1 of 49 female rats fed 1000 ppm, compared with 0/50 controls. A review of five studies of 2 years' duration

conducted with Sprague-Dawley rats at the same laboratory revealed that sarcomas of the subcutis occurred in control group males with the following frequencies: 2/102, 2/50, 2/50, 0/64 and 0/83. A review by Shell of unpublished literature revealed that in a 2-year study with Sprague-Dawley CD rats, spindle cell sarcomas were found in 3 of 48 male control rats (Piccirillo & Voelker, 1978). The historical incidence of spindle cell sarcomas ranged widely from 0% to 6%, indicating the variable spontaneous incidence of this type of lesion. Thus, although there are doubts regarding the relationship of these tumours to fenvalerate treatment, the possibility cannot be entirely excluded (Gordon & Weir, 1979). This study was considered to be inadequate for carcinogenicity assessment, as only one dose was tested; however, it provides valuable information for the overall evaluation of the carcinogenicity of fenvalerate.

In the study by Gordon & Weir (1979), different types of tumour might be diagnosed as the same type (spindle cell sarcoma) owing to the inappropriate method of tumour classification. Therefore, the tumours must be properly diagnosed on the basis of careful observation. To obtain the proper findings, the slides of the subcutaneous sarcomas sent from Litton Bionetics were examined histopathologically by a single pathologist (Ito, 1981). Upon re-examination, the tumours were not diagnosed as a group of spindle cell sarcomas, but as 1) a malignant schwannoma or perhaps a leiomyosarcoma, 2) a malignant mesenchymoma, 3) a fibrosarcoma and 4) two malignant amelanotic melanomas. Thus, the incidence of each sarcoma became very low (either 1 or 2 in 51 rats). There has been no attempt to reconcile these different diagnoses, which detracts from a ready acceptance of the re-evaluation. However, even in the original evaluation, there was no accumulation of sarcomas in female rats (and there is no reason to expect a sex difference in this respect). Furthermore, there was no evidence for an increase in these tumours in the Arai et al. (1980) study (see below) in which Wistar/SLC rats were administered fenvalerate at dietary concentrations up to 1500 ppm (equivalent to 75 mg/kg bw per day), which is 50% higher than in the current experiment. Thus, the observation of spindle cell sarcomas was not reproducible.

In a separate carcinogenicity study, groups of 80 male and 80 female Wistar/SLC rats were fed fenvalerate (S5602 technical, purity 93.4%) at a dietary concentration of 0, 50, 150, 500 or 1500 ppm (equivalent to 0, 2.5, 7.5, 25 and 75 mg/kg bw per day, respectively) for 104 (males) or 119 (females) weeks. Clinical signs were observed daily; body weight and feed consumption and water consumption were measured weekly; and ophthalmological examinations, urine analysis, haematology, blood chemistry, gross examinations, organ weights and histopathology were performed at termination.

No compound-related changes were observed in mortality, clinical signs, water intake, blood chemistry, haematology, urine analysis, eye examination or organ weights. Although the apparent dose-related decrease in mortality was observed in weeks 64 and 68, owing to respiratory disease, which spread more rapidly in the control than in the treated rats, final mortality of the treated groups was comparable to that of the control group. Body weight gains of males from the 500 and 1500 ppm groups and females from the 1500 ppm group were probably affected, although it was difficult to evaluate the male data exactly, owing to higher initial body weight of control males. The histopathological examination revealed giant cell infiltration in the spleen and liver at 1500 ppm and in lymph nodes and adrenals at 500 and 1500 ppm. Reticuloendothelial cell proliferation in the mesenteric lymph nodes was observed at the two highest doses. The neoplastic changes—namely, leukaemia, pituitary adenoma and phaeochromocytoma in adrenals—occurred in all the groups and in both sexes with similar incidence in treated and control groups and so were not related to the treatment. In females, uterine and mammary tumours were observed in all groups, including controls, with similar incidence and were not considered substance related.

The incidences of Leydig cell tumours were significantly increased in several groups, the overall values being as follows: 0 ppm, 21/75; 50 ppm, 36/78 ($P < 0.05$); 150 ppm, 27/79; 500 ppm, 56/76 ($P < 0.01$); and 1500 ppm, 53/77 ($P < 0.01$). In addition to these differences in Leydig cell tumour incidence, testicular atrophy and/or Leydig cell hyperplasia were seen in many males of all

groups. The incidence of testicular atrophy was slightly higher in treated groups than in the controls, and the incidence of Leydig cell hyperplasia was highest in the controls: 0 ppm, 19/75; 50 ppm, 12/78; 150 ppm, 7/79; 500 ppm, 10/76; and 1500 ppm, 3/77. The distinction between Leydig cell hyperplasia and Leydig cell adenoma is solely on the basis of size. As summarized in the esfenvalerate review by JMPR (Annex 1, references 95 and 97), according to some systems (e.g. the United States National Toxicology Program, as described by Boorman, Hamlin & Eustis, 1987), an adenoma is diagnosed if an area of hyperplasia is greater than a seminiferous tubule, whereas according to other systems (e.g. McConnell et al., 1992), an adenoma is diagnosed if it is equal to or greater than three seminiferous tubules. While such high incidences are common in Fischer 344 rats, this is not normally the case in Wistar rats. Indeed, a highly variable incidence of testicular tumours (11–100%) was observed at the time of the study in control Wistar/SLC rats from the same supplier over a 6-year period. Genetic analysis of Wistar/SLC rats and comparison with analysis of other strains in Japan indicate that two Wistar strains, one of which is Wistar/SLC, appear to be very similar to Fischer rats. The occurrence of Leydig cell adenomas is strongly age dependent. In the Wistar/SLC strain of rats, data from two reports from relevant time periods give the following total incidences: Experiment I (1974)—13 months, 10.0%; 19 months, 16.7%; 25 months, 100.0%; Experiment II (1974)—14 months, 14.0%; 20 months, 100.0%; 26 months, 987%. Although the final mortality percentages were generally similar across the groups in the current experiment (0 ppm, 79%; 50 ppm, 75%; 150 ppm, 72%; 500 ppm, 64%; 1500 ppm, 73%), there were higher numbers of rats at risk at 84 weeks (i.e. about the time that the rapid increase in incidence occurs) in the 500 and 1500 ppm groups, the mortality percentages at this time being as follows: 0 ppm, 65%; 50 ppm, 59%; 150 ppm, 55%; 500 ppm, 31%; 1500 ppm, 43%. This factor could have contributed to the between-group variations in adenoma incidence. It is also to be noted that in two studies described above, in which SD rats were fed diets containing fenvalerate at a concentration of 250 ppm (Gordon & Weir, 1978) or 1000 ppm (Gordon & Weir, 1979) for up to 2 years, there were no increases in Leydig cell tumours. It is concluded, therefore, that there may have been an accelerated growth rate of the Leydig cell proliferating lesions in some dose groups, but these cannot be interpreted as an increased emergence of tumours. It is concluded that fenvalerate is not carcinogenic. This study was considered to be adequate for the carcinogenicity assessment of fenvalerate in rats.

The NOAEL for systemic toxicity in this study was 150 ppm (equivalent to 7.5 mg/kg bw per day), based on reduced body weight gains and giant cell infiltration of lymph nodes and adrenals and reticuloendothelial cell proliferation in the lymph nodes at 500 ppm (equivalent to 25 mg/kg bw per day). There was no evidence of carcinogenic response, and the dietary no-effect level of fenvalerate was found to be 150 ppm (equivalent to 7.5 mg/kg bw per day) (Arai et al., 1980, 1981a).

2.4 Genotoxicity

The results of various mutagenicity studies (in vivo and in vitro) on fenvalerate are summarized in Table 7.

Table 7. Mutagenicity studies with fenvalerate

End-point	Test object	Concentration	Purity (%)	Results	Reference
In vitro					
Bacterial reverse mutation (Ames test) and rec assay	*Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1538 and *Bacillus subtilis* (H17 and M45)	1, 10, 100 and 1000 µg/plate (with and without S9 mix) or 10, 100, 1000 and 10 000 µg/disc (without S9 mix)	95	Negative	Suzuki & Miyamoto (1976)
Bacterial reverse	*S. typhimurium* strains TA98, TA100,	Up to 1 mg/plate (with and without S9 mix)	95	Negative	Suzuki & Miyamoto (1977);

End-point	Test object	Concentration	Purity (%)	Results	Reference
mutation (Ames test)	TA1535, TA1537 and TA1538				Suzuki, Kishida & Miyamoto (1979)

Table 7 (continued)

End-point	Test object	Concentration	Purity (%)	Results	Reference
Bacterial reverse mutation (Ames test)	*S. typhimurium* strains TA97, TA98, TA100 and TA1535	0, 10, 50, 100, 500, 1000, 2500 and 5000 µg/plate (with and without S9 mix)	97.6	Negative	Reynolds (1991a)
Fluctuation test and mammalian clastogenicity	*S. typhimurium* strains TA98 and TA100 and Chinese hamster (V79) cells	0, 1, 3 and 10 µg/ml and 0, 4, 20 and 40 µg/ml (with and without S9 mix)	99	Negative	Pluijmen et al. (1984)
Unscheduled DNA synthesis	Primary rat hepatocytes	0, 0.5, 5, 10, 50, 100, 500 and 1000 µg/ml	97.6	Negative	Bentley (1991)
In vivo					
Standard host-mediated assay	Mice	0, 60 and 125 mg/kg bw	95	Negative	Suzuki & Miyamoto (1976)
Standard host-mediated assay	Mice	0, 25 or 50 mg/kg bw	96	Negative	Brooks (1976)
Dominant lethal assay	Mice; male	0, 25, 50 and 100 mg/kg bw	96	Equivocal[a]	Dean (1975)
Bone marrow chromosomal aberrations	Chinese hamsters	0, 12.5 and 25 mg/kg bw	NS	Negative	Dean & Senner (1975)
Bone marrow micronucleus assay	Mice	0, 20, 38 and 75 mg/kg bw	97.6	Negative	Reynolds (1991b)
Bone marrow micronucleus assay	Mice	0, 100, 150 and 200 mg/kg bw, intraperitoneal	NS	Positive	Pati & Bhunya (1989)
Sex-linked recessive assay	*Drosophila melanogaster*	Adult 20 mg/l, larvae up to 50 mg/l or adult intraperitoneal 20 µg/ml	NS	Negative	Batiste-Alentorn et al. (1987)
Chromosomal aberration and SCE	Mice	0, 5, 10 and 20 mg/kg bw intraperitoneally	98.4	Weak clastogen; potent inducer of SCEs	Giri et al. (2002)
Micronucleus test in bone marrow cells	Mice	0, 2.5, 5, 10 and 20 mg/kg bw intraperitoneally	98.4	Positive	Giri, Giri & Sharma (2011)
		10 mg/kg bw	98.4	Negative	
Bone marrow micronucleus assay	Mice	0, 32.50, 75 and 150 mg/kg bw (single oral dose)	NS	Positive	Ghosh, Sharma & Talukder (1992)
Chromosomal aberrations test	Norway rats	Repeated gavage dose, 21 days	NS	Positive	Chatterjee, Talukder & Sharma (1982)

DNA, deoxyribonucleic acid; NS, not specified; S9, 9000 × *g* supernatant fraction of rat liver homogenate; SCE, sister chromatid exchange

[a] Significant reduction in viable fetuses, a significant increase in early fetal deaths occurring in females mated during the 4th week to males dosed at the highest level.

2.5 *Reproductive and developmental toxicity*

(a) Multigeneration studies

Rats

In a three-generation reproductive toxicity study, groups of Sprague-Dawley rats (11 male and 22 female rats per group) were administered fenvalerate (SD-43775, purity 98.0%) in the diet at a dose level of 0, 1, 5, 25 or 250 ppm (equivalent to 0, 0.07, 0.3, 1.7 and 16.7 mg/kg bw per day, respectively). The animals were fed for 9 weeks prior to mating and initiation of a standard three-generation (two litters per generation) reproduction study. Fertility, viability, gestation and lactating indices were calculated for each treatment group and compared with control values. All parental animals were necropsied. Ten male and 10 female weanlings from the F_{3b} litter were examined histologically at the conclusion of the study. Brain, heart, liver and kidney weights from F_{3b} litters were recorded. Several organs and tissues were examined microscopically.

The observations on the parental rats (F_0) reflected no effect related to treatment, although there was an unusually high frequency of skin lesions in treated and control rats. Necropsy findings on F_0 rats did not suggest any effect of the chemical, although the frequency of pulmonary tract lesions suggested that chronic respiratory disease was present in the rats without regard to treatment. In addition, enlarged adrenals were observed in most females. A review of historical data revealed that this was normal; considering the additional stress of pregnancy, they may have even been larger in comparison with the males. No remarkable effects were noted in F_0 parents at necropsy. No effect on reproductive parameters was seen in F_{1a} or F_{1b} litters, except for an apparent decreased fertility in females at 1 ppm. However, this effect showed no dose–response relationship in the higher treatment groups during the F_1 generation. The general appearance and behaviour of the parent rats of the second generation were judged to reflect no compound-related effect. The necropsy of the F_{1b} parents demonstrated a frequency of kidney changes (mottled, pale appearance) suggestive of a compound-related response (Table 8); there was no effect on reproductive parameters in F_{2a} or F_{2b} litters except for an apparent decrease in female fertility in the low-level (1 ppm) test group. As a dose–response relationship was not involved, this reduced reproductive capacity was not judged to be compound induced.

Table 8. Gross kidney changes in a multigeneration reproduction study in rats

Dietary concentration (ppm)	Gorss kidney changes in F_{1b} and F_{2b} rats			
	Males (F_{1b})	Females (F_{1b})	Males (F_{2b})	Females (F_{2b})
0	3	3	5	0
1	7	5	8	5
5	5	4	5	4
25	6	7	5	2
250	8	7	10	2

From Beliles, Makris & Weir (1978)

The mean body weights of the parents of the third-generation F_{2b} adults revealed a significant reduction at the high level (250 ppm) when control and treated groups were compared. The necropsy of F_{2b} parents revealed gross kidney changes similar to changes noted in the F_{1b} parents. However, the distribution with regard to dose was not judged to be consistent with a compound-related change. There was no effect on reproductive parameters in the F_{3a} or F_{3b} litters except for an apparent

decrease in female fertility in the low-level (1 ppm) test group. As a dose–response relationship was not involved, this reduced reproductive capacity was not judged to be compound induced. Histopathological examination of the F_{3b} weanlings was unremarkable.

With the exception of weight reduction in the third-generation parents, there was no effect of fenvalerate on any parameter measured in the study. The NOAEL for parental systemic toxicity was 25 ppm (equivalent to 1.7 mg/kg bw per day), based on reduced mean body weights seen at 250 ppm (equivalent to 16.7 mg/kg bw per day). The NOAEL for reproductive and offspring toxicity was 250 ppm (equivalent to 16.7 mg/kg bw per day), the highest dose tested (Stein, 1977; Beliles, Makris & Weir, 1978).

(b) Developmental toxicity

Mice

In a teratogenicity and reproduction evaluation study, groups of pregnant ICR mice (32–33 mice per group) were administered fenvalerate (S5602, purity 97.6%) at a dose level of 0, 5, 15 or 50 mg/kg bw per day on gestation days 6 through 15. On day 18, groups of 20–21 mice were sacrificed, and fetuses were removed and examined for somatic and skeletal abnormalities. About 12 females were allowed to deliver naturally, and the young were maintained for 3 weeks to weaning to evaluate postnatal deficits. In addition to the teratogenicity study, two male and two female weanlings from each dam were maintained for 8 weeks and mated to note any effects on their reproductive potential.

Toxic signs of poisoning were noted in maternal mice at the high dose level. Irregular respiration, hypersensitivity, tremors and salivation were noted after administration of the compound (first 30–60 minutes after dosing only). However, the symptoms were not seen the following morning and were not potentiated by consecutive treatment. There was no significant mortality over the course of the study, and no effects were noted on any of the animals as a result of continuous administration of fenvalerate. In fetal examinations, there were no somatic or skeletal changes noted in internal or external tissue evaluations. The mean fetal body weights were decreased at 5 mg/kg bw per day, but there was no dose–response relationship. The animals maintained in an abbreviated reproduction study showed no differences from control values in their ability to reproduce. There were no changes in the reproduction indices evaluated with any animals examined. Under the conditions of this bioassay, fenvalerate was shown to have no teratogenic potential in mice.

The NOAEL for maternal toxicity was 15 mg/kg bw per day, based on the clinical signs of toxicity seen at 50 mg/kg bw per day. The NOAEL for developmental toxicity was 50 mg/kg bw per day, the highest dose tested (Kohda, Kadota & Miyamoto, 1976a).

Rabbits

In a developmental toxicity study, groups of pregnant Dutch rabbits (group size varied from 20 to 31 rabbits) were administered fenvalerate (WL 43775, purity 97.0%) at 0, 12.5, 25 or 50 mg/kg bw per day from day 6 to day 18 of gestation. On day 28, the rabbits were sacrificed, and standard teratogenicity assessments were made with respect to early or late fetal death, viability and standard somatic and skeletal teratogenic potential.

Reduced body weight of dams at the highest dose level was observed. There were no significant differences from control values in any of the other parameters measured in the study. Under the conditions of the study, fenvalerate did not induce a teratogenic effect in rabbits.

The NOAEL for maternal toxicity was 25 mg/kg bw per day, based on reduced body weight of dams seen at 50 mg/kg bw per day. The NOAEL for developmental toxicity was 50 mg/kg bw per day, the highest dose tested (Van der Pauw et al., 1975).

2.6 Special studies

(a) Neurotoxicity

Hens

Groups of six hens were administered fenvalerate (purity 96.0%) orally in dimethyl sulfoxide (DMSO) at a dose level of 0 or 1000 mg/kg bw per day for 5 days. A positive control of tri-*ortho*-cresyl phosphate (TOCP; 0.5 ml/kg bw) was also included in the study. After 3 weeks, the fenvalerate and negative control animals were retreated using the same dosage regimen. At the conclusion of an additional 3-week observation period, animals were sacrificed, and histological examinations were performed on the central and peripheral nervous systems.

All animals receiving TOCP developed readily defined signs of delayed neurotoxicity, and histological lesions were seen in the sciatic nerve and spinal cord. Clinical signs and histopathological lesions were not noted in controls or fenvalerate-treated hens (Milner & Butterworth, 1977).

Mice and rats

In a special acute toxicity study, B6C3F1 mice and Sprague-Dawley rats showed the characteristic signs of intoxication following single oral doses of fenvalerate ranging from 56 to 320 mg/kg bw and from 133 to 1000 mg/kg bw, respectively. Neurological signs, such as splayed gait, tremors, ataxia and hindlimb incoordination, were observed at doses of 100 mg/kg bw or more (mice) and 133 mg/kg bw or more (rats) within 1–8 hours after dosing. These signs had disappeared in most animals within 72 hours. Slight peripheral nerve fibre damage was detected in surviving mice and rats sacrificed 10 days after dosing. The incidence and severity were dose related at doses above 56 and 180 mg/kg bw; however, even at lethal doses, there was no evidence of nerve lesions in some animals. Thus, two distinct neurological effects were observed: 1) a reversible ataxia and 2) incoordination plus a neuropathological effect manifested as sparse axonal damage in peripheral nerves (Parker et al., 1985).

Rats

A series of studies was performed to evaluate the neurotoxic potential (to induce axonal and myelin disruption) of a group of synthetic pyrethroid esters and of natural pyrethrum. Acute oral administration of fenvalerate, cypermethrin, resmethrin, permethrin and natural pyrethrum to rats at very high dose levels resulted in severe clinical signs of poisoning and mortality within 24 hours. Histopathological lesions were observed in the sciatic nerve with all compounds tested. At lower levels, fenvalerate (200 mg/kg bw), cypermethrin (100 mg/kg bw), permethrin (200 mg/kg bw) and pyrethrum (3500 mg/kg bw) did not show clinical signs of poisoning or histopathological lesions (Okuno, Kadota & Miyamoto, 1977a,b).

A histopathological examination was performed on the sciatic nerve and posterior tibial nerve of rats that had been exposed to acutely toxic levels of fenvalerate. After poisoning, and for 9 days during the course of recovery, axonal breaks, swelling and vacuolation, accompanied by phagocytosis of myelin, were seen. The degree to which myelin was disrupted was dose dependent and was closely associated with the acute signs of toxicity (Butterworth & Carter, 1976).

Groups of six male and six female rats were fed fenvalerate in the diet at a concentration of 0 or 2000 ppm for 8–10 days, after which the sciatic nerve was examined for histological signs of degeneration. All animals exposed to fenvalerate showed typical signs of acute intoxication, including ataxia, tremors and hyperexcitability. Histological examinations at the end of the feeding interval did not reveal any adverse effects of fenvalerate on the sciatic nerve (Hend & Butterworth, 1976).

In a study to evaluate the reversibility of the lesions induced in the sciatic nerve, rats were administered fenvalerate (purity 97.7%) in the diet at a dose level of 0 or 3000 ppm for 10 days. Mortality was evident, as 60% of the animals died within the course of the dietary treatment. Surviving animals were fed normal control diets and examined for 12 weeks following completion of

the feeding study. Animals were sacrificed every 3 weeks and examined histologically for sciatic nerve disruption. Sciatic nerves of rats on control diets sacrificed at 3 weeks continued to show swelling and disintegration of axons. At 6 weeks, there were no histological lesions. These results were also observed at the 9- and 12-week intervals, suggesting reversibility of the histopathological lesions observed following high-dose treatment with fenvalerate (Okuno, Kadota & Miyamoto, 1977b).

In a special study, fenvalerate was administered orally to Wistar or Carworth Farm E rats either as single doses or in the diet. When given in large quantities as a single dose of 250, 500, 800, or 1000 mg/kg bw, which were sufficient to kill some of the treated animals, fenvalerate produced sporadic Wallerian degeneration in the sciatic nerve. The neuropathy was never severe and was not seen in animals given the compound in sublethal doses. In feeding studies (for 5 weeks at 1000 mg/kg diet and for 3 months at 2000 mg/kg diet), no lesions were seen in the peripheral nerve, brain or spinal cord, and there was no evidence of cumulative neurotoxicity (Butterworth & Hend, 1976).

Groups of rats (4–6 of each sex per group) were administered fenvalerate orally at dose levels ranging from 0 to 400 mg/kg bw per day for 7 consecutive days. Mortality and clinical signs of acute poisoning were seen only at the highest dose level. A significant neurological deficit was demonstrated using an inclined plane test (expressed as the angle at which the animals cannot maintain their hold on an inclining plane). In addition to functional deficits, increases in β-glucuronidase and β-galactosidase activities in the posterior tibial nerve and trigeminal ganglia were observed. Functional motor deficits appeared to coincide with the administration of fenvalerate, reaching maximum effects between days 5 and 7 of treatment. Stimulation of lysosomal enzyme activity appears to coincide with neurological deficits and with sciatic nerve degeneration noted with acute (high level) intoxication (Dewar, Moffett & Sitton, 1977).

Hamsters

Groups of hamsters (five males and five females per group) were administered fenvalerate at a dose level of 0, 5, 10, 20 or 40 mg/kg bw per day for 5 consecutive days. In three separate experiments, dose-related increases in β-glucuronidase and β-galactosidase activities of peripheral nerves were observed. Minimal functional deficits were also noted during the treatment period and for 2 weeks following treatment. These data appear to be consistent and to be associated with axonal degeneration in the peripheral nerve (Dewar et al., 1978).

(b) Comparative neurotoxicity potential between fenvalerate and esfenvalerate

Mice

In a comparative study of esfenvalerate and fenvalerate, groups of B6C3F1 mice (12 of each sex per group) were fed diets containing 0, 50, 150 or 500 ppm esfenvalerate (lot no. PKG-85036, total purity of isomers 94.5%, of which 87.2% was the Aα isomer, esfenvalerate) for 3 months. An additional group of mice (12 of each sex) was fed 2000 ppm fenvalerate (lot no. 41028, total purity of isomers 95.5%, isomeric ratios Aα:Aβ:Bα:Bβ = 24.2:25.4:26.3:24.1), also for 3 months. Mean achieved concentrations in male mice were 0, 10.5, 30.5, 106 and 422 mg/kg bw per day at the dietary concentrations of 0, 50, 150, 500 and 2000 ppm, respectively. Mean achieved concentrations in female mice were 0, 12.6, 36.8, 113 and 462 mg/kg bw per day at the dietary concentrations of 0, 50, 150, 500 and 2000 ppm, respectively.

There were no deaths during the study. Clinical signs of toxicity were seen only in animals fed diets containing esfenvalerate at 500 ppm and fenvalerate at 2000 ppm. These were typical type II pyrethroid neurological responses, which were seen from the beginning and throughout the feeding period (i.e. salivation, hypersensitivity, fibrillation, tremor, convulsion, hunched posture and unsteady gait), as well as gross lesions, such as alopecia, skin scabs and sores. They were more severe in the mornings. Decreased body weight gain was seen in both sexes fed esfenvalerate at 500 ppm and fenvalerate at 2000 ppm. There were no consistent changes in feed consumption, and water intake was reduced only initially in the animals fed 500 ppm esfenvalerate and 2000 ppm fenvalerate. Urinary pH was decreased, whereas urinary protein, ketone, bilirubin, urobiligen and specific gravity were

increased, in animals fed esfenvalerate at 500 ppm and fenvalerate at 2000 ppm. In the same dose groups, haematological changes were found, which included decreased erythrocyte counts, haemoglobin concentration, packed cell volumes and/or mean cell volumes. Decreased total protein, glucose, phospholipid, cholesterol and triglyceride levels, with increased blood urea nitrogen, ALT, AST and LDH activities, were also observed in animals fed esfenvalerate at 500 ppm and/or fenvalerate at 2000 ppm. Males fed esfenvalerate at 150 ppm also showed decreased glucose and triglyceride concentrations. The major difference in blood biochemistry between esfenvalerate and fenvalerate was the LAP activity, which was decreased in animals fed esfenvalerate and increased in animals fed fenvalerate.

Skin lesions, thought to be due to scratching, were increased, along with, in some cases, enlargement of regional lymph nodes, at the high dose for both compounds. The salivary gland weights were increased in animals fed esfenvalerate at 500 ppm and fenvalerate at 2000 ppm. Compound-related histopathological changes were seen in the liver, spleen, lymph nodes, thymus, skin, kidney and stomach. There was a decrease in fat deposits in the liver and kidneys that was probably related to the decreased blood lipid levels. The animals dosed with fenvalerate also showed an increased incidence of microgranulomas and giant cell infiltration in the liver, spleen and lymph nodes. Very slight ulcerative changes in the glandular stomach were observed in four males receiving 500 ppm esfenvalerate, but not in females at the same dose, males and females at lower dose levels or any of the fenvalerate-treated mice. Examination of nervous tissue (brain, spinal cord and sciatic nerve) revealed no treatment-related tissue changes.

In summary, with the exception of the minor gastric ulcerations with esfenvalerate, there were no significant toxicological differences between mice fed esfenvalerate at 500 ppm and those fed fenvalerate at 2000 ppm. Esfenvalerate produced decreased LAP in the blood. Fenvalerate, but not esfenvalerate, at 500 ppm produced microgranulomatous changes in the liver, spleen and lymph nodes and increased LAP in the blood.

The NOAEL for esfenvalerate in mice in this study was 50 ppm in males (equal to 10.5 mg/kg bw per day), based on blood biochemical changes in male mice fed 150 ppm (equal to 30.5 mg/kg bw per day), and 150 ppm in females (equal to 36.8 mg/kg bw per day), based on blood biochemistry and overt toxic signs in female mice fed 500 ppm (equal to 113 mg/kg bw per day) (Koyama, 1985).

Rats

A comparative study of the neurotoxic potential of esfenvalerate (lot no. PKG-85036, purity 94.5%, 87.2% of which was Aα isomer, esfenvalerate) and fenvalerate (lot no. 41028, purity 95.5%, 24.2% of which was Aα isomer, esfenvalerate) was conducted in rats (8 of each sex per group except for the highest-dose groups, in which there were 16 of each sex). The compounds were administered in corn oil at a single oral dose of either esfenvalerate at 0, 5, 20 or 90 mg/kg bw or fenvalerate at 0, 20, 80 or 360 mg/kg bw, followed by observation for 2 weeks. Clinical observations, body weight measurements and functional testing using an inclined plane (slip angle test) were conducted during the observation period. At the end of this period, the rats were perfused with 10% buffered formalin, and the brain, spinal cord and sciatic/tibial nerves were examined histologically.

Eight animals, two males and one female receiving esfenvalerate at 90 mg/kg bw and one male and four females receiving fenvalerate at 360 mg/kg bw, were found dead within 24 hours after dosing. The toxic signs occurred within 2 hours of dosing and were the typical type II pyrethroid symptoms (i.e. muscular fibrillation, ataxia, tremors, salivation, limb paralysis, irregular respiration, hypersensitivity to sound, hunched posture and urinary incontinence). All neurological symptoms disappeared within 2 days of dosing. Muscular fibrillation, ataxia, salivation and/or hunched posture were seen in a few rats receiving esfenvalerate at 20 mg/kg bw and fenvalerate at 80 mg/kg bw. No toxic signs related to compound treatment were observed in any rat dosed with esfenvalerate at 5 mg/kg bw or fenvalerate at 20 mg/kg bw. Body weight depression was observed in males and females receiving fenvalerate at 80 and 360 mg/kg bw. The slip angle test did not reveal any functional deficit

in the treated groups. There were no treatment-related gross changes at necropsy. Slight to minimal axonal degeneration and/or demyelination with Schwann cell proliferation in peripheral nerves were recognized in many males and females treated with esfenvalerate at 90 mg/kg bw and fenvalerate at 360 mg/kg bw on histopathology. The incidences of these neural changes in the low- and mid-dose groups were comparable with those in controls. There were no remarkable differences in the incidence and severity of nerve damage between the high-dose groups of esfenvalerate and fenvalerate. The fact that these effects were not seen at the middle dose, although overt signs of toxicity were seen in both mid- and high-dose groups, suggests that the toxic signs are not related to peripheral nerve damage, but are more likely to be of pharmacological origin (Okuno, 1985).

(c) Antidotal studies

In an antidotal study, phenobarbital, pentobarbital and diphenylhydantoin were found to be effective in relieving the acute signs of intoxication caused by fenvalerate in rats. Phenobarbital (50 mg/kg bw, intraperitoneal) prevented tremor, diphenylhydantoin (100 mg/kg bw, intraperitoneal) reduced the toxic reaction and pentobarbital (35 mg/kg bw, intraperitoneal) removed the tremor reaction completely within 30 minutes. The combination of diphenylhydantoin with either of the barbiturates was effective in reducing the onset and severity of tremors, whereas a variety of other agents (delta-tubocurarine, atropine, meprobamate, diazepam, biperidin and trimethadione) were ineffective (Matsubara et al., 1977).

Fenvalerate administered to dogs at acute dosage rates sufficient to induce toxic signs of poisoning showed no consistent cardiovascular effects as measured by electrocardiogram. Respiratory stimulation was noted at high levels and was not reduced by anaesthetic supplements such as urethane, chloralose and pentobarbital (Kirkland & Albert, 1977).

The therapeutic potency of intraperitoneally administered methocarbamol was examined as an antidote against the acute oral intoxication of rats by a lethal dose of fenvalerate. Methocarbamol was initially administered at a dose of 400 mg/kg bw, followed by repeated doses of 200 mg/kg bw when tremors or hypersensitivity to sound were observed. Methocarbamol markedly decreased the mortality from 80%, which would be caused by an administration of 850 mg fenvalerate per kilogram body weight, to 0% and was effective in alleviating motor symptoms such as fibrillation, tremors, hyperexcitability, clonic seizures and choreoathetotic movements. A subcutaneous administration of atropine sulfate (25 mg/kg bw) was also effective in reducing the salivation produced by fenvalerate (Hiromori et al., 1986).

Effective treatments against fenvalerate-mediated effects have been investigated by quantifying behavioural skin sensory responses such as licking, scratching or biting of the treated sites by fenvalerate-treated guinea-pigs. Preparations containing vitamin E, corn oil or the local anaesthetic benzocaine were most effective (Malley et al., 1984).

(d) Pesticide interactions/synergistic effects

Intraperitoneal administration of *O*-ethyl-*O*-(4-nitrophenyl)phenylphosphonothioate or *S*,*S*,*S*-tributyl phosphorotrithioate to mice at 25 mg/kg bw increased the intraperitoneal toxicity of fenvalerate (administered 1 hour later) by more than 25-fold; the median lethal dose (LD_{50}) decreased from greater than 1000 mg/kg bw to 37 or 42 mg/kg bw. This study suggests that mammalian esterases highly sensitive to inhibition by certain organophosphorus compounds may play a critical role in fenvalerate detoxication. This kind of synergism among pesticides would be detrimental in increasing the toxicity of certain pyrethroids to mammals (Gaughan, Engel & Casida, 1980).

Esterases play a major role in the detoxification of many pyrethroid insecticides (Abernathy & Casida, 1973; Jao & Casida, 1974a; Soderlund & Casida, 1977a). Some inhibitors of "pyrethroid esterases" serve as synergists for pyrethroid toxicity to insects and mammals (Abernathy et al., 1973; Jao & Casida, 1974b; Soderlund & Casida, 1977b). Currently, the major use of pyrethroids is in cotton protection, where the crops may also be treated with esterase inhibitors (e.g. organophosphorus and methyl carbamate compounds).

Fenvalerate, when fed to adult rats for 14 days, did not induce hepatic microsomal enzyme, as measured by the comparative rates of deethylation of an organophosphate pesticide (chlorfenvinphos) (Creedy & Potter, 1976). Dietary levels of 1000 ppm did not induce the oxidative *O*-dealkylation that has been shown to be reflective of microsomal enzyme induction in rat liver.

The evaluation of several insecticides used on cotton for possible effects in altering the metabolism, toxicity and persistence of pyrethroids has been reported (Gaughan, Engel & Casida, 1980). Profenofos, *O*-ethyl-*O*-(4-nitrophenyl)phenylphosphonothioate and *S*,*S*,*S*-tributylphosphorotrithioate administered intraperitoneally to mice at 25 mg/kg bw strongly inhibited the liver microsomal esterases that hydrolyse fenvalerate and increased the intraperitoneal toxicity of fenvalerate by more than 25-fold.

(e) Behaviour

Guinea-pigs responded to dermal applications of fenvalerate by scratching the treated sites of the skin. This characteristic response was essentially over within 3–4 hours. When the powerful skin irritant oil of mustard was applied to fenvalerate-treated sites of skin 4–72 hours after the fenvalerate treatment, the behavioural skin sensory response was restimulated. Oil of mustard alone did not produce skin sensory stimulation. These results indicate that pyrethroid treatment causes a transient sensitivity to stimulation produced by chemical irritants (Malley et al., 1984).

To develop an animal model for studying skin sensory stimulation, Dunkin-Hartley guinea-pigs were treated with pyrethroid solutions on one side and control substances on the other side of their shaved backs. The animals responded by licking, scratching or biting the test sites, and activity was quantified by counting the number of times the animals responded. This behavioural activity reached a maximum 1–4 hours after treatment. A chemical irritant (oil of mustard) was able to restimulate the behavioural activity when applied within 24 hours after pyrethroid application. Skin sensory stimulation produced by cyano-containing pyrethroids, including fenvalerate, was significantly greater than that produced by non-cyano-containing pyrethroids. This behavioural model provides a quantitative means of evaluating pyrethroid non-erythematous skin sensory stimulation (Cagen et al., 1984).

(f) Endocrinological effects

The potential for fenvalerate and esfenvalerate to cause effects on the male endocrine system, primarily related to testicular tumorigenesis, was investigated. Groups of male Wistar/SLC rats were fed diets containing fenvalerate (lot no. 41103G, purity 92.9%) at a concentration of 0, 50, 150, 500 or 1500 ppm and esfenvalerate (lot no. 60610G, purity 86.0%) at a concentration of 375 ppm for 26 weeks. This mechanistic study included a component in which female rats were housed in close proximity (but without contact) to subgroups of the males from the low- and high-dose fenvalerate groups and the esfenvalerate group. Blood samples were taken at 4-week intervals.

No rats died during the course of the study. Lower body weights and decreased feed consumption relative to controls were observed in the 375 ppm esfenvalerate group and the 1500 ppm fenvalerate group. Neither serum luteinizing hormone (LH) nor serum testosterone concentrations were affected to any biologically significant extent; those statistically significant changes that were observed lacked either a dose–response relationship or temporal consistency. Marginal increases in relative (but not absolute) organ weights observed in the phase of the study where the males were housed separately from the females were as follows: testes (+5%), pituitary (+15%) and liver (+9%) for the 1500 ppm fenvalerate group; and epididymides (+4%), seminal vesicles (+9%) and liver (+4%) for the 375 ppm esfenvalerate group. There were no gross pathological or histopathological changes that were considered to be related to treatment. Housing males and females in close proximity without physical contact produced no effects in this study relative to males-only housing (Yamada, 1999).

In a published study, behavioural (open-field, stereotyped and sexual behaviours), physical (sexual maturation, body and organ weights) and endocrine (testosterone levels) parameters were evaluated in male offspring following maternal exposure during the prenatal and postnatal periods. In this study, pregnant Wistar rats received intraperitoneal injections of fenvalerate (purity not reported) at 10.0 mg/kg bw per day on gestation day 18, postnatal day (PND) 1 (during the first 10 minutes after delivery, before the dam started nursing) and daily on PNDs 2–5. Male offspring were evaluated daily for testis descent. On PND 120, a subset of male offspring was evaluated for sexual behaviour towards untreated ovariectomized females sexually activated with exogenous estradiol and progesterone. Stereotypical behaviour and motor activity in an open field were also assessed in the male offspring. Testosterone and organ weights of testis, seminal vesicles and ductus deferens were measured in a subset of male offspring sacrificed on PND 120.

The results of the study indicate that the treated male offspring had significantly ($P < 0.05$) reduced plasma testosterone levels (2.00 ± 0.29 ng/ml) compared with control male offspring (3.16 ± 0.24 ng/ml), significantly ($P < 0.05$) lower prostate weights (0.076 ± 0.006 g) when compared with control male offspring (0.103 ± 0.006 g) and significantly ($P < 0.05$) lower seminal vesicle weights (0.212 ± 0.020 g) when compared with control male offspring (0.299 ± 0.019 g). In sexual behaviour, treated male offspring had a significant increase in the total number of mounts until ejaculation (22.6 ± 2.77) compared with control male offspring (15.8 ± 1.1) and a significant decrease in the number of ejaculations during a 40-minute testing period (2.0 ± 0.3) when compared with control male offspring (3.0 ± 0.2). In the open-field study, male offspring had decreased periods of immobility compared with offspring from control dams. The study authors concluded that "these results indicate that perinatal exposure to fenvalerate during the critical periods of male brain sexual differentiation has long-term effects on the reproductive physiology and behavior of male rats" (Moniz et al., 1999).

In another study, behavioural (open-field, stereotyped and sexual behaviours), physical (sexual maturation, body and uterine weights) and neuroendocrine (estrous cycle and gonadal hormone levels) parameters were evaluated in female offspring following maternal exposure during the prenatal and postnatal periods. Pregnant Wistar rats received intraperitoneal injections of fenvalerate (purity not reported) at 10.0 mg/kg bw per day on gestation day 18, PND 1 (during the first 10 minutes after delivery, before the dam started nursing) and daily on PNDs 2–5. Female offspring were evaluated daily for vaginal opening. Beginning on PND 120, the estrous cycle of female offspring was assessed over a period of 15 days. On PND 120, a subset of female offspring was ovariectomized, stimulated with 17β-estradiol and progesterone, and evaluated for sexual behaviour towards experienced males. Stereotypical behaviour and motor activity in an open field were also assessed in female offspring. Progesterone and *p*-estradiol were measured in a subset of female offspring on PND 125.

The study results show that 1) sexual maturation was delayed, although body weight was unchanged until adulthood; 2) there was a reduction in sexual behaviour; 3) the estrous cycle was abnormal, and the uterine weight at different phases of the estrous cycle was modified; and 4) gonadal hormone levels in the plasma, stereotypy and open-field behaviours were not affected. The study authors attributed these results to an anti-estrogenic effect of perinatal exposure to fenvalerate during the critical periods of female brain sexual organization (Moniz et al., 2005).

In a study by Kaul et al. (1996), male ITRC bred Wistar rats received fenvalerate (purity 98.9%) in corn oil via intraperitoneal injections at 0, 100 or 200 mg/kg bw per day for a period of 45 days; the vehicle control group received corn oil (20 rats per group). Thyroid hormone (triiodothyronine [T_3] and thyroxine [T_4]) levels in blood were measured by radioimmunoassay. Intraperitoneal administration of fenvalerate in male rats for 45 days at a dose of 100 or 200 mg/kg bw per day induced hyperexcitability, tremors and paralysis. Tremors were observed after 7 days and gradually reached maxima on day 45. The symptoms were more marked in rats treated with 200 mg/kg bw per day. Fenvalerate provoked significant elevation of circulatory thyroid hormones, namely T_3 and T_4. When compared with controls, there was a statistically significant ($P < 0.001$) and

dose-dependent increase in both hormone levels at both doses, as shown in Tables 9 and 10. Significant increases in total calcium as well as protein-bound calcium in whole brain and hypothalamus were recorded.

Table 9. Effect of fenvalerate on serum T_3

Dose (mg/kg bw per day)	Serum T_3 level ± standard deviation (ng/ml)		
	15 days	30 days	45 days
0 (control)	0.41 ± 0.004	0.42 ± 0.003	0.40 ± 0.003
100	0.63 ± 0.009	0.92 ± 0.007	0.95 ± 0.004
200	1.00 ± 0.004	1.30 ± 0.006	1.51 ± 0.04

From Kaul et al. (1996)

Table 10. Effect of fenvalerate on serum T_4

Dose (mg/kg bw per day)	Serum T_4 level ± standard deviation (µg/dl)		
	15 days	30 days	45 days
0 (control)	43.01 ± 0.70	42.75 ± 0.95	42.00 ± 0.003
100	52.25 ± 0.86	60.50 ± 0.65	62.50 ± 0.004
200	60.00 ± 2.16	84.00 ± 1.83	93.00 ± 0.04

From Kaul et al. (1996)

The study authors concluded that the elevation of circulatory thyroid hormones as well as the active calcium pool could together be responsible for impairment of motor activity by altering various neuronal processes (Kaul et al.,1996).

In a published study, the effects of fenvalerate on female reproduction in rats whose mothers were exposed during gestation and lactation were evaluated. Pregnant Wistar rats were exposed to fenvalerate (40 mg/kg bw) or corn oil (vehicle) orally from gestation day 12 until the end of lactation.

Results showed decreases in ovarian weight, number of preantral follicles and number of corpora lutea at PND 75 and an increase in the resorption number when the fertility test was performed at PND 80. The study authors concluded that fenvalerate may impair the reproductive development of female offspring, manifested as reduced fecundity and ovulation number, resulting from the decrease in the number of corpora lutea (Guerra, de Toledo & Kempinas, 2011).

In a published study by Zhang et al. (2009), the effects of maternal fenvalerate (purity not reported) exposure on testicular development and spermatogenesis in ICR mice was evaluated. Pregnant mice received fenvalerate at 60 mg/kg bw per day in corn oil via gavage daily from PND 0 to weaning (PND 21); the vehicle control group received corn oil only. On PND 21, plasma testosterone was measured, and testes were weighed and subjected to histopathological examinations. On PND 80, another set of pups was sacrificed, plasma testosterone levels and testes weights were measured, epididymidal sperm were examined and testicular histopathology was conducted. To assess the impact of maternal exposure to fenvalerate on the fertility of male offspring, a third set of male pups was maintained to PND 80 and subsequently co-housed with untreated females.

Lactational fenvalerate exposure markedly decreased the absolute and relative weights of testes and increased the number of apoptotic cells in testes of pups at weaning. Histological examinations showed abnormal seminiferous tubules with large vacuoles or complete spermatogenic failure in testes of fenvalerate-treated mice at weaning. Additional experiments showed that lactational fenvalerate exposure markedly reduced messenger ribonucleic acid (mRNA) and protein levels of testicular P450scc, a testosterone synthesis enzyme. Consistent with down-regulation of testicular P450scc, the levels of serum and testicular testosterone at weaning were significantly decreased in pups whose mothers were exposed to fenvalerate during lactation. Although the

expression of testicular P450scc and serum and testicular testosterone levels in adulthood were restored to control levels, the decreased weights of testes and histological changes were irreversible. Importantly, the percentage of mature seminiferous tubules (stages VII and VIII) and the number of spermatozoa were obviously decreased in adult male mice whose mothers were exposed to fenvalerate during lactation. Taken together, these results suggest that lactational fenvalerate exposure permanently impairs testicular development and spermatogenesis (Zhang et al., 2009).

In a separate study, male mice were administered fenvalerate (60 mg/kg bw) by gavage daily from PND 35 to PND 63. Results showed that sperm count was significantly decreased in fenvalerate-treated mice. In addition, fenvalerate markedly decreased the layers of spermatogenic cells, disturbed the array of spermatogenic cells and increased the number of apoptotic cells in testes. The adverse effects of fenvalerate on male reproduction seemed to be associated with a decrease in serum and testicular testosterone levels. Although pubertal and early adult exposure to fenvalerate had little effect on the number of Leydig cells in testes, mRNA and protein levels of testicular biosynthetic enzymes, including P450 (17α) and P450scc, were significantly down-regulated in fenvalerate-treated mice.

The study authors concluded that pubertal and early-adult fenvalerate exposures induce a decrease in sperm counts in adulthood. The impairment of spermatogenesis is due to decreased testicular testosterone synthesis (Zhang et al., 2010a).

In a separate study, pregnant mice were administered fenvalerate (30 mg/kg bw) by gavage daily from gestation days 13 to 18. The weights of testes and epididymides were significantly decreased in mice whose mothers were exposed to fenvalerate during pregnancy. In addition, marked decreases in the number of mature seminiferous tubules (stages VII and VIII) were observed in the testes of adult male offspring. Fenvalerate also reduced the number of epididymal spermatozoa in adult male offspring. Serum testosterone level was decreased, and mRNA and protein levels of P450(17α) were significantly decreased in fetal testes.

The study authors concluded that the prenatal fenvalerate exposure irreversibly impairs testicular development and spermatogenesis, at least into early adulthood (Zhang et al., 2010b).

In a published study by Giray et al. (2010), effects of fenvalerate on thyroid hormone parameters in both healthy and selenium- and/or iodine-deficient rats were evaluated. In this study, 3-week-old male Wistar rats were administered fenvalerate (purity 92%) intraperitoneally. Eight groups of eight rats each were treated under various conditions. The treatment with fenvalerate had no effect on the T_4 levels of healthy controls, but caused significant increases in the T_4 levels of both iodine-deficient and selenium- plus iodine-deficient rats and a significant decrease in selenium-deficient rats. Fenvalerate treatment resulted in dramatic increases in the T_3 levels of all groups. Moreover, it caused an insignificant decrease in thyroid stimulating hormone in healthy controls, no effect in selenium-deficient rats and a significant elevation in iodine-deficient and selenium- plus iodine-deficient rats.

The study authors concluded that fenvalerate has the potential to significantly change thyroid hormone parameters in both normal and deficiency states, and the consequences of its modifying effect on thyroid status might be of critical importance, particularly in sensitive individuals and patients with thyroid dysfunction (Giray et al., 2010).

Fenvalerate was evaluated for its binding affinity with androgen receptor– and estrogen receptor–mediated mechanisms using short-term in vivo assays (Hershberger and uterotrophic). A 5-day Hershberger assay using castrated male rats measures the androgenic and antiandrogenic ability of the test substance. This test evaluates interaction of the test chemicals with the androgen receptor

present in several accessory glands or tissues (the ventral prostate, dorsolateral prostate, seminal vesicles with coagulating glands, and levator ani plus bulbocavernosus muscles). Fenvalerate (20, 40 or 80 mg/kg bw per day) was administered by oral gavage for 5 days to castrated male Crj:CD(SD)IGS rats (1 week after the castration, 11 weeks of age) with or without co-administration of testosterone propionate at 0.25 mg/kg bw per day (subcutaneous injection on the dorsal surface). Fenvalerate did not show any androgenic or antiandrogenic effects.

Potential effects of fenvalerate mediated through the estrogen receptor were evaluated by means of a 3-day uterotrophic assay using ovariectomized Crj:CD(SD)IGS rats (2 weeks after the ovariectomy, 8 weeks of age). No increase in weight of uterus (wet or blotted) was observed following oral exposure to fenvalerate (20, 40 or 80 mg/kg bw per day).

It was concluded by the study authors that, based on the results of these two reliable in vivo assays, fenvalerate did not exhibit any potential to cause adverse antiandrogenic or estrogenic effects at dose levels below those causing excessive systemic toxicity (Kunimatsu et al., 2002).

In a published study, the effects of fenvalerate on the reproductive system in rats were evaluated. Fenvalerate was administered orally at 0, 2, 4, 12 or 60 mg/kg bw to two groups of adult male SD rats. One group was sacrificed after 15 days of dosing, and the second group was sacrificed after 30 days of dosing.The levels of serum follicle stimulating hormone (FSH), LH, testosterone and testis homogenate testosterone were determined by radioimmunoassay. In addition, enzymes such as acid phosphatases and gamma-glutamyl transpeptidase (GGT) were examined, and sperm head counts were measured to explain the changes in daily sperm production.

In 15 days, serum FSH levels markedly increased in rats exposed to fenvalerate at or below 12 mg/kg bw ($P < 0.01$), and serum levels of LH were increased in the 12 mg/kg bw group ($P < 0.01$). In addition, testosterone levels in testis homogenates decreased after treatment with doses of 12 mg/kg bw or higher compared with the control group ($P < 0.01$). In 30 days, serum contents of FSH were significantly elevated at doses of 12 mg/kg bw or higher ($P < 0.01$), and homogenate levels of testosterone were diminished in the low-dose groups (2 and 4 mg/kg bw) ($P < 0.05$). Activity of acid phosphatases increased in the 12 and 60 mg/kg bw groups after 15 days ($P < 0.05$) and remained decreased in the high-dose group (60 mg/kg bw) after 30 days ($P < 0.05$), but the GGT level decreased dose dependently in the testis ($P < 0.05$). Fenvalerate caused a dose-dependent reduction in sperm head counts and daily sperm production, which were markedly reduced at doses of 12 mg/kg bw and above.

The study authors suggested that fenvalerate has obvious reproductive toxicity in male rats and can change the serum and testis homogenate levels of sex hormones or activity of testosterone, which may be correlated with the impairment of Sertoli cell and spermatogenic epithelium (Hu et al., 2002).

In a published study, the potential estrogenic activity of fenvalerate was evaluated in Wistar rats by examining reproductive and fertility capabilities. Adult male animals were treated for 30 days with fenvalerate at 20 or 40 mg/kg bw per day or corn oil (vehicle) by oral gavage. Further, a possible estrogenic activity of fenvalerate (0.4, 1, 4, 8 or 40 mg/kg bw) was tested after a 3-day treatment of immature female rats using the uterotrophic assay.

Exposure to the higher dose of fenvalerate was toxic to testis and epididymis, as shown by a decrease in the absolute weights and sperm counts in both organs. Although the sperm counts were reduced, fertility and sexual behaviour were similar in control rats and rats treated with 40 mg/kg bw. Fenvalerate did not exert estrogenic activity in vivo at the tested doses. Data suggest that fenvalerate treatment in this study failed to compromise fertility, possibly due to enhanced reproductive capacity in rodents compared with humans (Arena et al., 2008).

In a published study, fenvalerate was examined to evaluate whether pubertal fenvalerate exposure altered behavioural development. ICR mice were orally administered either vehicle or fenvalerate (purity not specified; 7.5 or 30 mg/kg bw per day) from PND 28 to PND 56. Learning and memory were assessed by the Morris water maze. Aggressive performance was evaluated by the aggressive behaviour test. Anxiety-related activities were detected by three tests: open-field, plus-maze and black–white alley. Sensorimotor function was analysed using beam walking and tightrope.

Results showed that the impairment of spatial learning and memory was more severe in fenvalerate-exposed female mice than in male mice. In addition, pubertal fenvalerate exposure inhibited aggressive behaviour in males. Moreover, pubertal fenvalerate exposure increased anxiety activities in females. The study authors concluded that, altogether, these results suggest that pubertal fenvalerate exposure impairs spatial cognition and behavioural development in a sex-dependent manner (Meng et al., 2011).

In a published study, pregnant rats were exposed to fenvalerate at 40 or 80 mg/kg bw per day or corn oil (vehicle, control) by oral gavage from gestation day 12 to lactation day 21 to evaluate reproductive, developmental and immunotoxic effects in male offspring. Immune and reproductive developmental effects were assessed in male offspring at PNDs 40 (peripuberty), 60 (post-puberty) and 90 (sexual maturity).

Treatment with the higher dose (80 mg/kg bw per day) resulted in convulsive behaviour, hyperexcitability and mortality in 45% of the dams. Fenvalerate was detected in the fetus due to placental transfer, as well as in pups due to breast milk ingestion, persisting in male offspring until PND 40, even though pesticide treatment was terminated on PND 20. However, fenvalerate did not produce marked alterations in age of testicular descent to the scrotum and preputial separation, parameters indicative of puberty initiation. In contrast, at puberty, there was a reduction in testicular weight and sperm production in male offspring of maternal treated rats. At adulthood, sperm counts and fertility did not differ between control and treated groups. Testosterone levels were not changed at any time during reproductive development. Similarly, no apparent exposure-related effects were detected in the histological structures of the lymphohaematopoietic system.

The study authors concluded that fenvalerate, in this experimental model, interfered with initial development of the male reproductive system, but that these effects on sperm production or fertility did not persist into adulthood (Nassr et al., 2010).

(g) Microgranulomatous lesions

A feeding study was conducted to examine the reversibility of the granulomatous changes in mouse liver, spleen and mesenteric lymph nodes, in which ddY strain mice were kept on a diet containing 0, 1000 or 3000 ppm fenvalerate for 6 weeks, followed by rearing on the control diet. Histopathological examination was conducted periodically. The granulomatous changes in liver, lymph nodes and spleen, which had been observed immediately and/or shortly after termination of the administration of fenvalerate, were lower in incidence as well as severity after 6 months of the recovery phase. Although these granulomatous changes were still observed at 12 months of the recovery phase, the incidence and severity were clearly reduced. Therefore, it was concluded that the granulomatous changes are not progressive and are reversible in nature (Ito et al., 1981).

In studies by Okuno et al. (1986a), male ddY mice were fed diets containing the [2*S*,α*S*]-, [2*S*,α*RS*]-, [2*R*,α*S*]- and [2*R*,α*R*]-isomers of fenvalerate at a dietary dose level of 0, 500 or 1000 ppm for 52 weeks, 500, 1000 or 2000 ppm for 52 weeks, 0, 125 or 1000 ppm for 13 weeks and 125 or 1000 ppm for 13 weeks. Microgranulomatous changes were observed in the mice treated with the [2*R*,α*S*]-isomer after 1, 2 or 3 months. In contrast, the changes did not occur in mice treated with the [2*R*,α*R*]-isomer under the same conditions. Neither [2*S*,α*S*]- nor [2*S*,α*RS*]-isomers caused microgranulomatous changes at 500 or 1000 ppm after 1 year. To clarify the causative agent of granuloma formation, the cholesterol ester of CPIA, a lipophilic conjugate from the [2*R*,α*S*]-isomer of fenvalerate, was injected

intravenously into ddY mice. Microgranulomatous changes were observed in the liver of mice treated with the [2*R*]-, [2*S*]-, or [2*RS*]-CPIA–cholesterol ester 1 week after a single treatment of 1, 10 or 100 mg/kg bw, as well as in the liver of mice treated with a single dose of 10 or 30 mg/kg bw of the [2*R*]-CPIA–cholesterol ester and kept up to 26 weeks afterwards. Histochemical examination and microscopic autoradiography of the liver demonstrated the presence of tritium, derived from ^{3}H-labelled [2*R*]-CPIA and cholesterol in giant cells and Kupffer cells. Another histochemical examination showed the presence of cholesterol ester in the liver of mice treated with the [2*R*,α*S*]-isomer. These results support the hypothesis that the CPIA–cholesterol ester is the causative agent of the microgranulomatous changes induced by fenvalerate.

In further studies by Okuno et al. (1986b), male and female ddY mice were fed diets containing technical fenvalerate (0, 10, 30, 100 or 300 ppm in the diet for 20 months or 0, 100, 300, 1000 or 3000 ppm in the diet for 17–18 months). Microgranulomatous changes were observed in the lymph nodes, liver and spleen, the NOAEL for such changes being 30 ppm. To examine the reversibility of these changes, ddY mice (males and females) were fed a diet containing technical fenvalerate at a dose level of 1000 or 3000 ppm for 6 weeks, followed by a control diet for up to 12 months. The size and number of the microgranulomatous changes were reduced with time. These changes were typical of foreign body granulomas and did not have the appearance of granulomas formed in response to an immunological stimulus. In order to further elucidate the fate of the granulomatous changes, mice were similarly treated with 10 or 30 ppm of the [2*R*]-CPIA–cholesterol ester and sacrificed at 1, 4 or 8 weeks after injection. Microgranulomatous changes, including microgranulomas and giant cell infiltrations, were seen in each case. Giant cells were seen clearly at the later stages, whereas microgranulomas were more evident at the early stages. The presence of the microgranulomas 8 weeks after injection is suggestive of foreign body microgranulomas.

To exclude hypersensitivity as the cause of the microgranulomatous changes previously observed, groups of five female BALB/cA/nu/nu/SLC nude mice were fed dietary racemic fenvalerate at 1000 or 3000 ppm in the diet for 4 weeks. Histopathological changes typical of the fenvalerate-induced microgranulomas were observed in all of the treated mice. This study indicates that granulomatous changes induced by fenvalerate are not mediated by hypersensitivity reactions (Miyamoto et al., 1984).

More recently, a dose-related incidence of hepatic microgranulomas was found in groups of six male and six female Beagle dogs fed fenvalerate at 0, 250, 500 or 1000 ppm in the diet for 6 months. Histiocytic cell infiltration of mesenteric lymph nodes also occurred in female dogs fed 500 or 1000 ppm and in males fed 1000 ppm. Multinucleated cells were occasionally seen. The reversibility of these effects was not studied (Parker et al., 1984).

2.7 Studies on metabolites and degradates

The major photodegradation product of fenvalerate, SD 54597, decarboxyfenvalerate [2-(3-phenoxyphenyl)-3-(4-chlorophenyl)-4-methylpentanenitrile], is less acutely toxic to mammals than fenvalerate itself. It is also a plant metabolite. The results of acute toxicity studies on decarboxyfenvalerate are summarized in Table 11.

Groups of Fischer 344 rats (30 of each sex per group) were fed diets containing decarboxyfenvalerate (lot no. not stated, purity 98%) at a concentration of 0, 30, 100, 300, 3000 or 10 000 ppm for up to 13 weeks. Mortality was not affected by treatment, but body weight gains were significantly reduced in males of the 10 000 ppm group throughout the study and in females during the first 7 weeks. Body weight gains were also significantly reduced in males of the 3000 ppm group during the first 7 weeks. White blood cell counts were significantly reduced at weeks 7 and 13 in both sexes of the 10 000 ppm group and in females of the 3000 ppm group ($P < 0.05$, Dunnett's test). Reductions in white blood cell counts in males of the 100 and 300 ppm groups at 13 weeks were not dose related. Mean corpuscular volume was significantly reduced at 7 and 13 weeks in both sexes of the 3000 and 10 000 ppm groups ($P < 0.05$, Dunnett's test), except for females of the 3000 ppm group at 7 weeks. Some deviations in blood chemistry values were noted, but these were generally inconsistent or lacking sufficient magnitude to be of biological importance. Absolute and relative (to

body weight) liver weights were increased in both sexes of the 300 ppm group and higher, except for males of the 300 ppm group at 7 weeks. Consistent, significant increases were also seen in absolute or relative kidney weights in both sexes of the 10 000 and 3000 ppm groups. Significant microscopic changes were limited to centrilobular hepatocellular hypertrophy in both sexes of the 10 000 ppm group, minimal focal areas of hepatocellular necrosis in some rats of the 10 000 and 3000 ppm groups and an increased incidence, but not severity, of glomerulonephrosis in both sexes of the 10 000 ppm group at 13 weeks.

Table 11. Acute toxicity of decarboxyfenvalerate (SD 54597, plant metabolite)

Species	Strain	Sex	Route	Vehicle	Result	Reference
Rat	Sprague-Dawley	M, F	Oral	Corn oil	> 5000 mg/kg bw	Cannelongo (1983a)
Rabbit	New Zealand White	M, F	Dermal	Deionized water	> 2000 mg/kg bw	Cannelongo (1983b)
	New Zealand White	M, F	Dermal	Undiluted	Not a skin irritant	Cannelongo (1983c)
	New Zealand White	M, F	Eye	Undiluted	Not an eye irritant	Cannelongo (1983d)
Guinea-pig Buehler test	Dunkin-Hartley	M, F	Skin	Undiluted	Not a skin sensitizer	Parker (1983)

F, female; M, male

The NOAEL for this study was 300 ppm (equivalent to 30 mg/kg bw per day), based on reduced body weight gains in males during the first 7 weeks, a decrease in white blood cell count in females, reduced mean corpuscular volume in both sexes, increases in liver and kidney weights, and hepatocellular necrosis in both sexes at 1000 ppm (equivalent to 100 mg/kg bw per day) (Parker et al., 1986).

In a teratology study, decarboxyfenvalerate (purity 98%) was dissolved in corn oil and administered by gastric intubation to groups of 30 sperm-positive COBS CD (SD) BR albino rats at a dose of 0, 30, 300 or 3000 mg/kg bw per day on gestation days 6–15. Control dams received corn oil only.

Few clinical signs were observed in any treatment group throughout gestation, and no statistically significant differences were noted in maternal body weights, body weight gains, numbers of corpora lutea, implantations or resorptions, pregnancy rates or implantation rates among all groups. However, the maternal carcass weights at sacrifice (maternal body weight minus the weight of the gravid uterus) were statistically significantly decreased and the maternal relative liver weights were increased in the high-dose group.

No fenvalerate-related adverse effects were observed in the pups in terms of reduction in mean fetal weights or increased incidences of malformations, anatomical variations or resorptions. The malformations and variations observed in this study included those that are commonly seen among rats, such as short ribs, misaligned sternebrae, hydronephrosis and hydroureter. Only two severely malformed fetuses were observed: one was a control with cleft palate, cleft lip, absence of the tongue and a short lower jaw; the other was an animal in the low-dose group that exhibited umbilical hernia, short limbs and fused digits.

The NOAEL for maternal toxicity was 300 mg/kg bw per day, based on decreased body weights and increased relative liver weights seen at 3000 mg/kg bw per day. The developmental toxicity NOAEL was 3000 mg/kg bw per day, the highest dose tested (Lu, 1983).

Acute intraperitoneal toxicity studies were conducted on fenvalerate metabolites in mice. Mice were treated with test compound at various dose ranges using corn oil as the vehicle (except PBacid, which was dissolved in DMSO). Treated mice were observed for 14 days. Gross pathological examination was conducted at termination. The results of the acute toxicity tests are summarized in Table 12.

Table 12. Acute intraperitoneal toxicity of fenvalerate metabolites in mice

Chemical	Purity (%)	LD_{50} (mg/kg bw)	
		Males	Females
Fenvalerate	97.6	88.5	85
CPIA	> 99	351	350
PBalc	> 99	371	424
4′-OH-PBalc	> 99	750–1000	750–1000
2′-OH-PBalc	> 99	876	778
PBacid	97.3	154	169
4′-OH-PBacid	99	783	745
2′-OH-PBacid	99	859	912
3-Phenoxybenzaldehyde	99	415	416
Sodium thiocyanate	99	604	578

From Kohda et al. (1979)

CPIA, 2-(4-chlorophenyl)isovaleric acid; LD_{50}, median lethal dose; 2′-OH-PBacid, 3-(2′-hydroxyphenoxy)benzoic acid; 4′-OH-PBacid, 3-(4′-hydroxyphenoxy)benzoic acid; 2′-OH-PBalc, 3-(2′-hydroxyphenoxy)benzyl alcohol; 4′-OH-PBalc, 3-(4′-hydroxyphenoxy)benzyl alcohol; PBacid, 3-phenoxybenzoic acid; PBalc, 3-phenoxybenzyl alcohol

The mutagenic potential of two fenvalerate metabolites in bacteria has been investigated. Neither COOH-fenvalerate (lot nos CTS94001 and A1-1M, purities 96.1% and 91.3%, respectively) nor $CONH_2$-fenvalerate (lot no. CTS94002, purity 98.4%) induced mutations in *Salmonella typhimurium* strain TA98, TA100, TA1535 or TA1537 or in *Escherichia coli* WP2*uvrA* in the presence or absence of an exogenous metabolic system (Yamada & Kitamoto, 1995a,b).

It can be concluded that the minor metabolites of fenvalerate are all less toxic than the parent compounds.

3. Observations in humans

3.1 Occupational studies

In a Swedish study, occupational exposure to three products containing synthetic pyrethroids used in forestry to protect conifer seedlings against the large brown pine weevil (*Hylobius abietis*) was studied. Fenvalerate was used in one product and permethrin in two products containing a mixture of *trans*/*cis* isomers in the proportions 60/40 and 75/25, respectively. A high frequency of symptoms was reported in connection with the use of fenvalerate, which led to a withdrawal of the product for use in forestry. Some symptoms were also reported in groups exposed to permethrin. For example, in interviews carried out with 139 planters, irritative symptoms of the skin and upper

respiratory tract were reported in 73% of users for fenvalerate, 63% for permethrin (*trans*/*cis* 75/25) and 33% for permethrin (*trans*/*cis* 60/40), respectively (Kolmodin-Hedman, Swensson & Akerblom, 1981, 1982).

Among 23 workers exposed to synthetic pyrethroids, including fenvalerate, 19 experienced one or more episodes of abnormal facial sensation developing between 30 minutes and 3 hours after exposure and persisting for 30 minutes to 8 hours. None of the subjects had experienced symptoms on any other part of the body. The sensations were described as “tingling”, “burning”, “like coming from the cold” and “nettle rash”. However, there were no abnormal neurological signs, and electrophysiological studies showed normal responses in the arms and legs. It was concluded that the facial symptoms were probably due to spontaneous firing of sensory nerve fibres or sensory nerve endings whose threshold has been transiently lowered by the exposure to a pyrethroid. The localization of symptoms, their time of onset and duration probably depend on the local concentration of pyrethroid, which is influenced by factors affecting absorption through the skin locally (Le Quesne, Maxwell & Butterworth, 1980).

A field study of 16 workers engaged in the agricultural application of fenvalerate associated exposure with cutaneous symptomatology. Paraesthesia usually developed at exposed body sites after several hours, and the symptoms then progressed from a mild itch to a stinging sensation and peaked with numbness. Sweating, exposure to sun or heat or the application of water to the exposed site aggravated these symptoms. Normal sensation returned with 24 hours of cessation of exposure (Tucker & Flannigan, 1983).

No clinical case of pyrethroid poisoning had been reported until outbreaks of acute deltamethrin and fenvalerate poisoning occurred among cotton growers in China in 1982. Having been told (in error) that pyrethroids were non-toxic, the farmers handled the pyrethroid insecticides without taking any precautions. After repeated spraying in the cotton fields, the mild cases presented with severe headaches, dizziness, fatigue, nausea and anorexia, with transient changes in the electroencephalogram, while a severe case developed muscular fasciculation, repetitive discharges in the electromyogram and frequent convulsions. However, all were found by follow-up studies to have completely recovered, and the prognosis of acute pyrethroid poisoning proved to be correct (He, 1987).

More recently, the same author reviewed 573 cases of acute pyrethroid poisoning reported in the Chinese medical literature during 1983–1988. Among these were 196 cases of acute fenvalerate poisoning, 63 of which were occupational, due to inappropriate handling, and 133 accidental, mostly due to ingestion. Two died of convulsions. All others recovered with symptomatic and supportive treatment within 1–6 days. A comprehensive review of clinical manifestations was included (He et al., 1989).

3.2 Mode of action

The toxicity of fenvalerate and other pyrethroids has been reviewed by Aldridge (1990), Vijverberg & Van den Bercken (1990) and Appel & Gericke (1993). In all species tested, high doses of pyrethroids induced toxic signs that are characteristic of a strong excitatory action on the nervous system (Vijverberg & Oortgiessen, 1988). The principal action of pyrethroids on the peripheral nervous system is to induce pronounced repetitive activity. In particular, sensory organs produce trains of nerve impulses instead of single impulses, both in vitro and in vivo. Depending on the particular pyrethroid, sensory nerves, motor nerve endings and muscle fibres may also show repetitive activity. Another possible effect is depolarization of membranes, leading to increased release of neurotransmitters or even blockage of excitation.

The pyrethroids can be divided into two classes by the pattern of toxic signs shown in rats after the administration of sublethal and/or lethal doses. The early synthetic pyrethroids induced signs of intoxication in rats very similar to those of the natural pyrethrins. Initial tremors, aggressive behaviour and extreme sensitivity to sensory stimuli were followed by prostration, with whole-body tremors preceding death. This sequence, later designated T syndrome, is characteristic of non-cyano

pyrethroids and may be incomplete, depending on the molecular structure and on the route of application and dose of the pyrethroid. Most of the α-cyano-3-phenoxybenzyl pyrethroids evoke a distinct sequence of symptoms, called the CS syndrome. Initial pawing and burrowing behaviour is rapidly followed by profuse salivation and whole-body tremors, progressing to sinuous writhing (choreoathetosis), which gradually becomes more intense. In the final stage, clonic seizures may occur. As in the case of non-cyano pyrethroids, symptoms may be evoked or enhanced by sensory stimuli.

Vijverberg & Van den Bercken (1990) summarized the evidence for the hypothesis that the main biological activity of pyrethroids is mediated by effects on sodium channels. The stereoselective pyrethroids fix themselves onto the sodium channel and cause them to stay open much longer than normal, resulting in prolongation of the transient current associated with membrane depolarization. In frog myelinated nerve fibres, this prolongation can range from 6 milliseconds with phenothrin to 1770 milliseconds with deltamethrin. The time constant is a characteristic of each structure, and those causing the T syndrome and those causing the CS syndrome in rats can generally be distinguished on this basis. Investigations into the site of action of pyrethroids within the nervous system have not resulted in a clear distinction between central and peripheral effects. Several published results suggest that in rats, the lethal site of pyrethroids is located peripherally, possibly in the cardiovascular or respiratory system. These effects can be attributed to modifications of presynaptic and postsynaptic sodium channels. Postsynaptic neurotransmitter responses are unaffected by concentrations of pyrethroids that cause marked sodium channel modification. At high concentrations, insecticidal as well as non-insecticidal pyrethroid isomers cause a nonspecific suppressive effect on the postsynaptic neurotransmitter response.

3.3 Clinical studies

Fenvalerate was administered dermally to the skin of the arm or face of adult men and women at dose levels of approximately 20–40 mg. Control applications were carried out on the same individuals, and subjective evaluations were performed with respect to dermal irritation. There was no erythema or other visible skin effects, and an evaluation of the subjective responses suggested no significant differences between the fenvalerate-treated and control portions of the body (Hine, 1976).

A double-blind study utilizing 29 male volunteers was performed to test the skin reaction of formulated fenvalerate. The emulsifiable concentrate formulation was diluted with water and administered to one side of the face, on the cheek, with a control formulation applied to the opposite cheek. There were no signs of dermatitis noted at 24 hours following administration. Subjective analyses of irritation or skin sensation were performed with each individual. Under the conditions of the study, the formulation of fenvalerate did not produce abnormal skin sensations. There were no indications that any of the symptoms noted (which included tingling, burning, stinging, itching, swelling, numbness or heat) was associated with fenvalerate (Brown & Slomka, 1979).

A double-blind study was performed to compare human discrimination of technical fenvalerate, the heavy-ends fraction of distilled fenvalerate, and ethyl alcohol (vehicle) applied to the lower edge of each earlobe of 36 adult (both male and female) volunteers on three separate occasions. Both forms of fenvalerate caused a statistically significant increase in paraesthesia, compared with the vehicle alone. The onset of the cutaneous sensations occurred 1 hour after application, peaked at 3–6 hours and lasted approximately 24 hours. Numbness, itching, burning, tingling and warmth were the most frequently reported sensations. The difference between the effects of the two fractions of fenvalerate was not statistically significant (Knox, Tucker & Flannigan, 1984).

A clinical study was conducted to evaluate the difference in the degree of paraesthesia induced by a number of pyrethroids. Applications of 0.05 ml fenvalerate formulated to field strength (0.13 mg/cm^2) were made to a 4 cm^2 area of earlobe on five occasions, the opposite earlobe receiving distilled water. Participant evaluation after each application continued for 48 hours and involved description of the cutaneous sensations. Each participant was treated after each application with one of the remaining compounds. Fenvalerate (like the other pyrethroids) induced skin sensations. The paraesthesia developed with a latency period of approximately 30 minutes, peaked by 8 hours and

deteriorated as early as 24 hours. The local application of dl-α-tocopheryl acetate markedly inhibited the occurrence of skin sensations (Flannigan et al., 1985a,b; Malley et al., 1985).

3.4 Summary

In summary, fenvalerate has been found to induce skin sensations in some of the workers who handle this insecticide. Clinical studies showed that the skin sensations develop with a latency period of approximately 30 minutes, peak by 8 hours and deteriorate after 24 hours. Numbness, itching, tingling and burning are symptoms frequently reported. α-Tocopheryl acetate has been found to inhibit the occurrence of these skin sensations.

Comments

Biochemical aspects

Metabolism studies have been conducted in rats, mice and dogs using ^{14}C-labelled esfenvalerate and fenvalerate. Fenvalerate was rapidly absorbed in these mammals, widely distributed to organs and tissues and rapidly metabolized. Excretion of an isomeric mixture of fenvalerate and esfenvalerate was very rapid in rats and mice, with 63–94% of the administered label being excreted within 1 day after oral dosing. Approximately equal quantities of radioactivity were eliminated in the urine and faeces. Tissue residue concentrations were generally very low, with residue levels being lower in mice than in other species. Fenvalerate and its esters concentrate in adipose tissue, adrenal gland, intestinal mucosa, skin and hair. The cyano moiety remains in the body (particularly in skin and hair) longer than other components. A placental transfer study of fenvalerate and esfenvalerate in rats indicated that there was virtually no transfer of radioactivity from maternal blood to the fetus and no evidence of accumulation in the fetus or amniotic fluid. In dogs administered fenvalerate orally, the total recovery of radioactivity in excreta was less than that in mice or rats, but the elimination half-life was similar to those found in the rodents (0.5–0.6 day).

Fenvalerate undergoes several major metabolic reactions, including cleavage of the ester linkage, hydroxylation in the acid and alcohol moieties and conversion of the cyano group to thiocyanate and carbon dioxide. The pattern of hydroxylation was different in rats and dogs, and the glycine conjugate, 3-phenoxybenzoylglycine, was the major conjugate of the alcohol moiety in dogs, whereas it was a minor one in rats. The proportions of glucuronides formed at the acid moiety and their hydroxy derivatives were also greater in dogs. There were no major sex differences in the metabolism of fenvalerate.

In a 28-day feeding study in mice, the major metabolites in the liver and kidney of animals fed [^{14}C-chlorophenyl]esfenvalerate and [^{14}C-chlorophenyl]fenvalerate were CPIA and the hydroxylated derivative of CPIA. These disappeared after administration of untreated diets. In addition, CPIA–cholesterol ester was found in mice fed [^{14}C-chlorophenyl]fenvalerate, but not in mice fed [^{14}C-chlorophenyl]esfenvalerate.

Photolytic degradation on plants can produce a decarboxylated fenvalerate not known to occur in mammals.

Toxicological data

Clinical signs, such as choreoathetosis (coarse tremors progressing to sinuous writhing), sedation, salivation, dyspnoea and/or clonic seizures and sometimes body tremors and prostration, were observed in acute studies. These signs, which are typical of a type II pyrethroid, have been observed in various mammalian species tested with either esfenvalerate or fenvalerate and are characteristic of a strong excitatory action on the nervous system.

The oral LD_{50} in rats was greater than or equal to 451 mg/kg bw (vehicle dependent), whereas in mice, it was greater than or equal to 100 mg/kg bw (vehicle dependent). The dermal LD_{50} in rats was 5000 mg/kg bw. The inhalation LC_{50} in rats was greater than 101 mg/m^3 (3-hour exposure). No

data are available on skin and eye irritation or skin sensitization. However, data are available on esfenvalerate, which is not irritating to the skin and minimally irritating to the unwashed eyes of rabbits. It was a skin sensitizer in guinea-pigs using the Magnusson and Kligman maximization test, but was not a sensitizer using the Buehler test method.

In a 90-day dietary toxicity study of fenvalerate in rats, the NOAEL was 125 ppm (equivalent to 12.5 mg/kg bw per day), based on increased relative kidney and liver weights at 500 ppm (equivalent to 50 mg/kg bw per day). Although survival was reduced at 2000 ppm (equivalent to 200 mg/kg bw per day), the highest dose tested in this study, no degeneration of the sciatic nerve was observed.

The overall NOAEL in dogs fed fenvalerate for 3 or 6 months was 250 ppm (equivalent to 18.8 mg/kg bw per day), based on hepatic multifocal microgranulomas and histiocytic infiltration of the mesenteric lymph nodes in females at 500 ppm (equivalent to 37.5 mg/kg bw per day).

Three long-term dietary studies of fenvalerate toxicity and carcinogenicity in mice have been reported. In the first study, the NOAEL was 300 ppm (equivalent to 15 mg/kg bw per day), based on reduced body weight gain, clinical signs of hyperactivity, mortality, clinical chemistry changes and microscopic changes in liver, kidney and mesenteric lymph nodes observed at 1000 ppm (equivalent to 50 mg/kg bw per day). In the second study, the NOAEL was 10 ppm (equivalent to 1.5 mg/kg bw per day), based on an increased incidence of microgranulomatous changes in mesenteric lymph nodes and other visceral and peripheral lymph nodes observed at 50 ppm (equivalent to 7.5 mg/kg bw per day). In the third study, the NOAEL was 30 ppm (equal to 3.48 mg/kg bw per day), based on slightly decreased erythrocyte counts, decreased serum glucose concentrations and increased histiocytic infiltration (liver and lymph nodes) and granulomatous changes in the liver and lymph nodes at 100 ppm (equal to 12.3 mg/kg bw per day). The overall NOAEL for long-term dietary exposure of mice to fenvalerate was 30 ppm (equal to 3.48 mg/kg bw per day), based on histopathology in various organs, but most consistently in lymph nodes, at 50 ppm (equivalent to 7.5 mg/kg bw per day).

There was no evidence for carcinogenicity from any of these studies in mice at fenvalerate concentrations up to 3000 ppm (equivalent to 150 mg/kg bw per day).

In a published oral gavage study of carcinogenicity of fenvalerate in mice, no evidence for carcinogenicity was observed at the highest dose of 80 mg/kg bw per day.

Four chronic toxicity and carcinogenicity studies of fenvalerate have been performed in rats, but two of the studies are inadequate for the assessment of carcinogenic potential.

Groups of Sprague-Dawley rats were administered diets containing fenvalerate at either 0 or 1000 ppm (equivalent to 50 mg/kg bw per day) for 2 years. No group difference in the incidence of any specific tumours was reported.

In a second 2-year study in rats (Wistar/SLC strain), there were observations of body weight gain depressions and giant cell infiltration of lymph nodes and adrenals and reticuloendothelial cell proliferation in the lymph nodes at 500 ppm (equivalent to 25 mg/kg bw per day).

The overall NOAEL for long-term toxicity of fenvalerate in rats was 150 ppm (equivalent to 7.5 mg/kg bw per day), on the basis of body weight gain reduction in males and giant cell infiltration of lymph nodes and adrenals and reticuloendothelial cell proliferation in the lymph nodes at 500 ppm (equivalent to 25 mg/kg bw per day). In males, Leydig cell atrophy, Leydig cell hyperplasia and Leydig cell adenomas were significantly increased in some groups, but there was no clear dose–response relationship. Similar to the Fischer 344 strain rats, Leydig cell adenomas are particularly common and variable in the Wistar/SLC strain (which is not to be confused with the Wistar strain, in which incidences of these tumours are low). Consequently, variation in the incidence of this tumour type cannot be used with any confidence in carcinogenicity evaluation. There was no evidence of carcinogenicity in rats at doses up to 1500 ppm (equivalent to 75 mg/kg bw per day), the highest dose tested.

The Meeting concluded that fenvalerate was not carcinogenic in mice or rats.

Fenvalerate was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. No evidence of genotoxicity emerged from the in vitro assays, but inconsistent results were obtained in the male mouse dominant lethal assays and the in vivo cytogenetic assays.

Available data allowed the Meeting to conclude that fenvalerate is unlikely to be a DNA reactive compound, but no firm conclusion could be reached on its in vivo clastogenicity.

On the basis of the absence of carcinogenicity in mice and rats and the absence of DNA reactivity, the Meeting concluded that fenvalerate is unlikely to pose a carcinogenic risk to humans at expected dietary levels.

In a three-generation reproduction study in rats, the NOAEL for parental toxicity was 25 ppm (equivalent to 1.7 mg/kg bw per day), based on reduced mean body weights seen at 250 ppm (equivalent to 16.7 mg/kg bw per day). The NOAEL for reproductive and offspring toxicity was 250 ppm (equivalent to 16.7 mg/kg bw per day), the highest dose tested.

In a developmental toxicity study in mice, the NOAEL for maternal toxicity was 15 mg/kg bw per day, based on irregular respiration, hypersensitivity, tremors and salivation after administration of the compound (first 30–60 minutes after dosing only) seen at 50 mg/kg bw per day. The NOAEL for developmental toxicity was 50 mg/kg bw per day, the highest dose tested.

In a developmental toxicity study in rabbits, the NOAEL for maternal toxicity was 25 mg/kg bw per day, based on reduced body weight of dams seen at 50 mg/kg bw per day. The NOAEL for developmental toxicity was 50 mg/kg bw per day, the highest dose tested.

The Meeting concluded that fenvalerate is not teratogenic in mice or rabbits.

A study of the neurotoxic potential of esfenvalerate and fenvalerate in corn oil was conducted in rats following a single oral gavage dose. The NOAELs were 5 and 20 mg/kg bw for esfenvalerate and fenvalerate, respectively, based on the toxic signs typical of type II pyrethroids. Signs were observed within 2 hours of dosing at 20 and 90 mg/kg bw for esfenvalerate and fenvalerate, respectively.

No histopathological lesions in the sciatic nerve were observed in rats following a single-dose administration of fenvalerate at 200 mg/kg bw. In a separate study, rats were administered fenvalerate orally at dose levels ranging from 0 to 400 mg/kg bw per day for 7 consecutive days. A significant neurological deficit was demonstrated using an inclined plane test (expressed as the angle at which the animals cannot maintain their hold on an inclining plane). In addition to functional deficits, increases in the activity of the lysosomal enzymes β-glucuronidase and β-galactosidase in the posterior tibial nerve and trigeminal ganglia were observed.

Fenvalerate did not cause delayed neuropathy in hens at 1000 mg/kg bw per day for 5 days.

Several published studies are available that evaluate the effects of fenvalerate on sexual maturation, stereotyped and sexual behaviour, hormonal measurements, sperm measurements and changes in various organ weights and histopathology in mice and rats. These studies were conducted following single or multiple oral administrations of fenvalerate. Generally, reproductive parameters and neurobehavioural parameters were affected at about 20 mg/kg bw per day via oral doses.

The acute intraperitoneal toxicity study results indicate that fenvalerate metabolites were less toxic than the parent compound fenvalerate.

The major photodegradation product of fenvalerate, decarboxyfenvalerate, or 2-(3-phenoxyphenyl)-3-(4-chlorophenyl)-4-methylpentanenitrile, was evaluated for its acute toxicity; results indicated that it was less toxic than the parent compound. In a 90-day toxicity study in rats with decarboxyfenvalerate, the NOAEL was 300 ppm (equivalent to 30 mg/kg bw per day), based on reduced body weight gains in males during the first 7 weeks, a decrease in white blood cell count in females, reduced mean corpuscular volume in both sexes, increases in liver and kidney weights, and hepatocellular necrosis at 1000 ppm (equivalent to 100 mg/kg bw per day). In a developmental toxicity study with decarboxyfenvalerate in rats, the NOAEL for maternal toxicity was 300 mg/kg bw

per day, based on decreases in body weights and increases in relative liver weights seen at 3000 mg/kg bw per day. The developmental toxicity NOAEL was 3000 mg/kg bw per day, the highest dose tested.

Observations in humans indicate that fenvalerate causes the transient facial sensations that appear to be common to pyrethroids, particularly those possessing a cyano group.

The Meeting concluded that the existing database on fenvalerate was adequate to characterize the potential hazards to fetuses, infants and children.

Toxicological evaluation

The Meeting reaffirmed the ADI of 0–0.02 mg/kg bw on the basis of a parental systemic toxicity NOAEL of 1.7 mg/kg bw per day observed in the three-generation reproduction study in rats, based on reduced mean body weights seen at 16.7 mg/kg bw per day and using a safety factor of 100. This ADI was supported by the NOAEL of 3.5 mg/kg bw per day observed in the long-term toxicity and carcinogenicity studies in mice, based on the slight decrease in erythrocyte counts, increased histiocytes and granulomatous changes in the liver and lymph nodes (mesenteric, visceral and peripheral) at 7.5 mg/kg bw per day.

The Meeting established an acute reference dose (ARfD) of 0.2 mg/kg bw on the basis of the NOAEL of 20 mg/kg bw observed in the single oral dose neurotoxicity study in rats, based on clinical signs of toxicity (muscular fibrillation, ataxia, salivation and/or hunched posture) seen at 90 mg/kg bw and using a safety factor of 100. This ARfD was supported by the developmental toxicity study in mice in which the NOAEL was 15 mg/kg bw per day, based on irregular respiration, hypersensitivity, tremors and salivation after administration of the compound (first 30–60 minutes after dosing) seen at 50 mg/kg bw per day.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen-month to 2-year studies of toxicity and carcinogenicity[a,b]	Toxicity	30 ppm, equal to 3.5 mg/kg bw per day	50 ppm, equivalent to 7.5 mg/kg bw per day
		Carcinogenicity	3000 ppm, equivalent to 150 mg/kg bw per day[c]	—
	Developmental toxicity study[d]	Maternal toxicity	15 mg/kg bw per day	50 mg/kg bw per day
		Embryo and fetal toxicity	50 mg/kg bw per day[c]	—
Rat	Two-year studies of toxicity and carcinogenicity[a,b]	Toxicity	150 ppm, equivalent to 7.5 mg/kg bw per day	500 ppm, equivalent to 25 mg/kg bw per day
		Carcinogenicity	1500 ppm, equivalent to 75 mg/kg bw per day[c]	—
	Three-generation study of reproductive toxicity[a]	Reproductive toxicity	250 ppm, equivalent to 16.7 mg/kg bw per day[c]	—
		Parental toxicity	25 ppm, equivalent to 1.7 mg/kg bw per day	250 ppm, equivalent to 16.7 mg/kg bw per day
		Offspring toxicity	250 ppm, equivalent	—

Species	Study	Effect	NOAEL	LOAEL
			to 16.7 mg/kg bw per day[c]	
	Neurotoxicity study (single dose)[d]	Neurotoxicity	20 mg/kg bw	90 mg/kg bw
Rabbit	Developmental toxicity study[d]	Maternal toxicity	25 mg/kg bw per day	50 mg/kg bw per day
		Embryo and fetal toxicity	50 mg/kg bw per day[c]	—
Dog	Thirteen-week and 6-month studies of toxicity[a,b]	Toxicity	250 ppm, equal to 18.8 mg/kg bw per day	500 ppm, equal to 35.5 mg/kg bw per day

[a] Dietary administration.
[b] Two or more studies combined.
[c] Highest dose tested.
[d] Gavage administration.

Estimate of acceptable daily intake for humans

0–0.02 mg/kg bw

Estimate of acute reference dose

0.2 mg/kg bw

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposures

Critical end-points for setting guidance values for exposure to fenvalerate

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid and complete
Dermal absorption	Not available
Distribution	Widely distributed
Potential for accumulation	None
Rate and extent of excretion	Rapid and complete (half-life 0.5–0.6 day)
Metabolism in animals	Extensive
Toxicologically significant compounds in animals, plants and the environment	Parent compound and decarboxylated fenvalerate
Acute toxicity	
Rat, LD_{50}, oral	≥ 451 mg/kg bw (vehicle dependent)
Rat, LD_{50}, dermal	5000 mg/kg bw
Rat, LC_{50}, inhalation	> 101 mg/m^3 (3 h)
Rabbit, dermal irritation	Not available
Rabbit, ocular irritation	Not available
Dermal sensitization	Not available

Short-term studies of toxicity	
Target/critical effect	Nervous system, clinical signs
Lowest relevant oral NOAEL	12.5 mg/kg bw per day (rat)
Lowest relevant dermal NOAEL	1000 mg/kg bw per day (rabbit)
Lowest relevant inhalation NOAEC	7 mg/m^3 (rat)
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Multiple target organs
Lowest relevant NOAEL	3.5 mg/kg bw day (mouse)
Carcinogenicity	Not carcinogenic in mice or rats
Genotoxicity	
	Not DNA reactive, inconsistent results in in vivo cytogenetic assay
Reproductive toxicity	
Target/critical effect	None
Lowest relevant reproductive NOAEL	16.7 mg/kg bw per day (highest dose tested)
Lowest relevant parental NOAEL	1.7 mg/kg bw per day
Lowest relevant offspring NOAEL	16.7 mg/kg bw per day (highest dose tested)
Developmental toxicity	
Target/critical effect	None
Lowest relevant maternal NOAEL	15 mg/kg bw per day (mouse)
Lowest relevant embryo/fetal NOAEL	50 mg/kg bw per day (mouse and rabbit)
Neurotoxicity	
Target/critical effect	Clinical signs typical of type II pyrethroids
Acute neurotoxicity NOAEL	20 mg/kg bw (rat)
Subchronic neurotoxicity	No data
Other toxicological studies	
Studies on metabolites	Metabolites/degradation products less toxic than parent
Immunotoxicity	No data
Medical data	
	Transient facial sensations in humans

Summary

	Value	Study	Safety factor
ADI	0–0.02 mg/kg bw	Three-generation reproduction study (rat)	100
ARfD	0.2 mg/kg bw	Single-dose study (rat)	100

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FLUXAPYROXAD

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Explanation

Fluxapyroxad is the International Organization for Standardization (ISO)–approved name for 3-(fluoromethyl)-1-methyl-*N*-(3′,4′,5′-trifluoro[1,1′-biphenyl]-2-yl)-1H-pyrazole-4-carboxamide (International Union of Pure and Applied Chemistry) (Chemical Abstracts Service No. 907204-31-3). It is a fungicide that belongs to the carboxamide class. Its proposed fungicidal mode of action is inhibition of succinate dehydrogenase, resulting in the inhibition of the citric acid cycle and mitochondrial electron transport pathways.

Fluxapyroxad has not been evaluated previously by the Joint FAO/WHO Meeting on Pesticide Residues and was reviewed at the present Meeting at the request of the Codex Committee on Pesticide Residues.

All the critical studies contained certificates of compliance with good laboratory practice (GLP).

Evaluation for acceptable daily intake

1. Biochemical aspects

The absorption, distribution, metabolism and excretion, as well as the toxicokinetics, of fluxapyroxad have been investigated in Wistar rats. Summaries of the relevant data are presented below.

1.1 Absorption, distribution and excretion

The absorption, distribution, excretion and metabolism of fluxapyroxad in Wistar rats were investigated using the active substance radiolabelled in the aniline ring or in the 4-position of the pyrazole ring. The test item was a mixture of either [^{14}C]fluxapyroxad (pyrazole label) and unlabelled fluxapyroxad or [^{14}C]fluxapyroxad (aniline label) and unlabelled fluxapyroxad (Figure 1).

Figure 1. Radiolabelled forms of fluxapyroxad used in absorption, distribution, metabolism and excretion studies: structure and position of labels

Structural formula of [^{14}C-aniline-U]fluxapyroxad

Structural formula of [^{14}C-pyrazole-4]fluxapyroxad

From Fabian & Landsiedel (2009a)

Biokinetic studies with [^{14}C]fluxapyroxad were performed to investigate plasma kinetics, mass balance, tissue distribution and urinary, faecal and biliary excretion (Table 1). [^{14}C]Fluxapyroxad was administered to male and female Wistar rats (four of each sex per dose) via oral gavage in a water/0.5% carboxymethylcellulose/1% Cremophor vehicle. Plasma kinetics were first characterized by administering a single dose of 5, 50 or 500 mg/kg body weight (bw) and measuring plasma concentrations at 1, 2, 4, 8, 24, 48, 72, 96, 120, 144 and 168 hours. The times to reach maximum plasma concentrations (T_{max}) were 24 hours (500 mg/kg bw), 8 hours (50 mg/kg bw) and 1 hour (5 mg/kg bw) in both sexes, with mean maximum plasma concentrations (C_{max}) of 65.31, 13.35 and 1.85 µg equivalents (Eq) per gram in males and 66.08, 11.83 and 1.57 µg Eq/g in females, respectively. Areas under the plasma concentration–time curve (AUCs) were 4215.2, 434.6 and 45.4 µg Eq·h/g in males and 5667.4, 532.4 and 35.7 µg Eq·h/g in females, respectively. Importantly, no sex differences in the rate or extent of absorption were observed. Also, AUCs scaled with dose, indicating that absorption was not saturated and was most probably the result of passive diffusion. Although the rates of elimination were the same in males and females in the time intervals following the T_{max}, they were slightly reduced in females in the time interval immediately following the T_{max}. This could be due to the slight difference in tissue distribution between males and females and would explain the slightly higher AUCs in females at doses of 150 and 500 mg/kg bw and the difference in the bile excretion pattern (Fabian & Landsiedel, 2009a).

Table 1. Dosing groups within biokinetics study with [^{14}C]fluxapyroxad

Test group	Dose of labelled material (mg/kg bw)	Number of rats of each sex	Remarks
Blood/plasma level experiments			
Experiment 1	500	4	Retro-orbital blood sampling at 1, 2, 4, 8, 24, 48, 72, 96, 120 and 144 h; exsanguination at 168 h
Experiment 2	50	4	
Experiment 3	5	4	
Balance/excretion experiments			
Experiment 4	150	4	Metabolism cages used to sample urine at 6, 12, 24, 48, 72, 96, 120, 144 and 168 h and excreta at 24, 48, 72, 96, 120, 144 and 168 h
Experiment 5	7.5	4	Expired air collected from two males in experiments 4 and 5 for 48 h
Experiment 6	150 (14 × ^{12}C, 1 × ^{14}C)	4	
Tissue distribution experiments			
Experiment 7	150	12	Sacrifice times: 150 mg/kg bw: 16, 48, 72 and 96 h (male rats); 16, 56, 80 and 104 h (female rats)
Experiment 8	7.5	12	7.5 mg/kg bw: 1, 8, 28 and 48 h (male rats); 1, 20, 28 and 48 h (female rats) Three rats of each sex per time point Tissue analysis, total radioactivity
Excretion via bile			
Experiment 9	150	4	Bile was collected at 3 h intervals for up to 72 h
Experiment 10	7.5	4	Urine and faeces were collected at 24 h intervals until 72 h Sacrifice time: 72 h; selected tissue analysis; total radioactivity

From Fabian & Landsiedel (2009a)

[^{14}C]Fluxapyroxad doses of 150 and 7.5 mg/kg bw were chosen for mass balance, tissue distribution and biliary excretion studies, based on the results of the plasma kinetic studies and repeated-dose toxicological studies. The duration of these studies (time to sacrifice) was based on the T_{max}: ½ T_{max}, ¼ T_{max} and ⅛ T_{max} in each sex. In a tissue distribution study with single high and low doses, [^{14}C]fluxapyroxad at 150 mg/kg bw was administered orally (by gavage) to four rats of each sex per dose, with males sacrificed at 16, 48, 72 and 96 hours and females sacrificed at 16, 56, 80 and 104 hours. The highest tissue concentrations of radioactivity in males and females were found at 16 hours. Radioactivity was widely distributed in both sexes with a similar pattern: the highest concentrations were found in the gut contents and stomach contents. However, lower concentrations were found in numerous other organs and tissues, including the liver, thyroid, adrenal glands, kidney, pancreas, testes/uterus and brain. For males and females, radioactivity declined in all tissues over time. In the single low-dose study, [^{14}C]fluxapyroxad at 7.5 mg/kg bw was administered orally (by gavage) to four rats of each sex per dose, with males sacrificed at 1, 8, 28 and 48 hours and females sacrificed at 1, 20, 28 and 48 hours after the administration of the test substance. A similar pattern of distribution of the radiolabel was observed as in the high-dose group. Radioactivity declined in all tissues over time for the males and females except for the gut contents, in which radioactivity increased 3-fold in males from 1 to 8 hours prior to decreasing. A similar effect occurred in gut contents in females, in which radioactivity increased about 1.5-fold from 1 to 20 hours up to 1.7-fold at 28 hours prior to decreasing at 48 hours.

Mass balance and excretion were investigated in males and females in single-dose studies with [^{14}C]fluxapyroxad at 150 or 7.5 mg/kg bw in animals (four animals of each sex per dose) with or without bile catheterization. Excreta and bile were collected in cannulated animals up to 72 hours, whereas excreta were collected in uncannulated animals up to 168 hours. A subset of uncannulated animals (two of each sex per dose) was monitored in metabolism cages equipped to measure ^{14}C in expired air. A repeated-dose study was also performed in which males and females (four of each sex) were administered 14 doses of unlabelled fluxapyroxad one per day on days 1–14 and [^{14}C]fluxapyroxad on day 15 with monitoring up to 168 hours after dose administration. Mean total recoveries of radioactivity were greater than 89% in males and females for all dose groups. For all dose groups, the majority of the administered radioactivity was excreted via faeces (84.43–94.30% after 168 hours). The total amount of radioactivity excreted in urine after 168 hours was in the range of 3.47–16.7%. Non-relevant portions of the administered radioactivity were excreted as carbon dioxide in exhaled air. The time course of the amount of radioactivity found in urine and faeces indicated that the excretion occurred predominantly within 3 days after dosing, indicating low potential for bioaccumulation. Bile duct cannulation experiments showed that the bile was a major route of excretion. Within 72 hours after administration of [^{14}C]fluxapyroxad at a dose level of 150 mg/kg bw, excretion via bile was found to be 58.86% and 63.19% of the administered radioactivity in males and females, respectively. Within 72 hours after administration of [^{14}C]fluxapyroxad at a dose of 7.5 mg/kg bw, excretion via bile was found to be 50.92% and 55.80% of the administered radioactivity in males and females, respectively. These data also demonstrated a sex-independent excretion pattern for [^{14}C]fluxapyroxad.

Based on the amounts of radioactivity excreted via bile and urine, as well as the radioactive residue found in cage wash and carcass, the bioavailability of [^{14}C]fluxapyroxad in rats was calculated to be about 65% and 67% of the applied dose for male and female rats at a dose of 150 mg/kg bw and about 68% and 80% of the applied dose for males and females at a dose of 7.5 mg/kg bw.

After a single oral application of 150 mg/kg bw, 84–92% of the applied radioactivity was excreted (including cage washings). The majority of radioactivity was excreted via faeces, accounting for 86.4% and 80.8% (pyrazole label) and 82.4% and 77% (aniline label) of the administered dose. In urine, only 4.3–6.5% (aniline label) and 4.2–8.6% (pyrazole label) of the applied radioactivity were detected. Overall, no significant sex-specific differences were observed regarding the route and rate of excretion (Table 2).

Radioactivity levels were determined in liver, kidney, fat and plasma at 1 and 16 hours after dosing. These two time points correspond to the two maximum plasma levels found in the biokinetics study. The results are summarized in Table 3.

In general, no significant sex-specific differences were found, with the exception of residues in fat, which were higher in females than in males. After a single oral dose of 7.5 mg/kg bw, radioactivity levels were highest in the liver, accounting for 5.32% (males) and 5.26% (females) of the applied dose. The lowest levels were detected in plasma, with 0.19% and 0.22% of the applied dose for males and females, respectively. In kidney, 0.43–0.63% of the administered dose was detected. The radioactivity in fat was 0.46–2.78% of the administered dose. At 150 mg/kg bw, radioactivity levels were lower than those at 7.5 mg/kg bw, accounting for 1.30–1.63% of the administered dose in liver, 0.09–1.14% of the administered dose in kidney, 0.39–1.78% of the administered dose in fat and 0.04–0.16% of the administered dose in plasma.

1.2 Biotransformation

The metabolic fate of [^{14}C]fluxapyroxad was investigated in a follow-up study. Material from the biokinetics study was used for investigation of metabolite patterns in urine and faeces (dose groups B, C and D) and in bile (dose groups R and S). Three additional groups of animals (designated DX, V and W) were dosed with [^{14}C]fluxapyroxad specifically for the metabolism study (Table 4). Urine, plasma and bile, as well as extracts of faeces, liver, fat and kidney tissue, were analysed by high-performance liquid chromatography. Individual metabolites were detected down to a level of about 0.1% of the administered dose or less. Metabolites were identified by liquid chromatography

with mass spectrometry or tandem mass spectrometry. The structures and codes of identified fluxapyroxad metabolites are shown in Appendix 1 (Schopfer & Labib, 2009).

Table 2. Excretion balance at 168 hours post-dosing with a single high dose of 150 mg/kg bw

	% of administered dose			
	Group DXLP Pyrazole label		Group DXLA Aniline label	
	Males	Females	Males	Females
Urine				
0–24 h	2.64	4.36	2.27	2.23
24–48 h	1.01	2.59	1.36	2.88
48–72 h	0.20	0.61	0.39	0.96
72–96 h	0.18	0.54	0.16	0.21
96–168 h	0.16	0.50	0.09	0.19
Subtotal	4.20	8.59	4.27	6.47
Faeces				
0–24 h	47.36	30.04	50.36	25.85
24–48 h	29.58	37.19	23.58	34.86
48–72 h	6.85	9.37	6.29	12.43
72–96 h	1.68	2.94	1.48	2.56
96–168 h	0.87	1.27	0.70	1.33
Subtotal	86.34	80.80	82.42	77.04
Cage wash	1.69	0.93	0.19	0.46
Total	92.23	90.32	86.88	83.97

From Fabian & Landsiedel (2009a)

Table 3. Distribution of radioactivity in plasma and selected tissues at T_{max} of plasma level (1 or 16 hours)

	% of administered dose			
	Group V Single low dose (7.5 mg/kg bw) Pyrazole label T_{max} = 1 h		Group W Single high dose (150 mg/kg bw) Pyrazole label T_{max} = 16 h	
	Males	Females	Males	Females
Liver	5.32	5.26	1.30	1.63
Kidney	0.43	0.63	0.09	0.14
Fat	0.46	2.78	0.39	1.78
Plasma	0.19	0.22	0.04	0.16

From Fabian & Landsiedel (2009a)

In urine, 23 metabolites were identified. The main metabolites were in the range of 0.2–8.6% of the applied dose and identified to be M700F004, M700F005/M700F024 (detected only in male rats), M700F009/M700F028, M700F014, M700F015 and M700F061 (detected only in female rats) (Tables 5 and 6). For all dose groups and both sexes, no amounts of the unchanged parent compound were detectable at any of the time intervals investigated.

Table 4. Dosing groups for metabolism study with [^{14}C]fluxapyroxad

Test group	Dose of labelled material (mg/kg bw)	Number of each sex	Remarks
Group B (Experiment 5)	7.5	4	Metabolism cages used to sample excreta and expired air Sacrifice and tissue collection after 168 h
Group C (Experiment 6)	150 (14 × ^{12}C, 1 × ^{14}C)	4	
Group D (Experiment 4)	150	4	
Group R (Experiment 10)	7.5	4	Bile collected at 3 h intervals for up to 72 h
Group S[a] (Experiment 9)	150	4	
Group DXLA[b]	150	4	Metabolism cages used to collect excreta Sacrifice at 168 h (male and female rats)
Group DXLP[b]		10	
Group V[c]	7.5	4	Liver, kidney, fat and plasma collected after 1 h
Group W[c]	150	4	Liver, kidney, fat and plasma collected after 16 h

From Schopfer & Labib (2009)
[a] Because of insufficient bile flow, two experiments (SF and SFW) were conducted.
[b] DXLA: aniline label; DXLP: pyrazole label.
[c] Dose groups V and W were dosed with the pyrazole label.

Table 5. Proportions of metabolites identified in rat urine (biokinetics study)

Compound	% of administered dose					
	Group B Single low dose (7.5 mg/kg bw)		Group D Single high dose (150 mg/kg bw)		Group C Repeated high dose (14 + 1 × 150 mg/kg bw)	
	Males (0–120 h)	Females (0–120 h)	Males (0–96 h)	Females (0–120 h)	Males (0–120 h)	Females (0–120 h)
M700F004	1.61	0.63	0.30	0.39	0.58	0.53
M700F005/M700F024	1.12	ND	0.76	ND	0.82	ND
M700F009/M700F028	1.03	ND	0.51	ND	0.42	ND
M700F009	ND	3.70	ND	3.34	ND	3.47
M700F011	0.05	0.14	0.04	0.08	0.18	0.08
M700F014	3.09	8.60	0.40	2.26	0.99	0.56
M700F015	1.41	0.45	0.40	0.17	2.14	0.48
M700F016	0.37	0.07	0.10	0.02	0.04	0.36
M700F020	0.13	0.20	0.04	0.05	0.27	0.05
M700F023	0.03	ND	0.01	ND	ND	ND
M700F025	ND	ND	ND	ND	ND	ND
M700F026/M700F027	ND	ND	ND	ND	ND	ND
M700F061	ND	0.63	ND	1.22	ND	1.95
Total identified	8.83	14.42	2.57	7.52	5.44	7.48

From Schopfer & Labib (2009)
ND, not detected/identified

In faeces, the unchanged parent compound was found to be the main constituent of the extracts at high dose levels (i.e. dose groups D, C and DX) for both labels (19–44% of the dose applied) and represented only a minor part of the extractable radioactivity (3% of the dose applied) at

the low dose level (dose group B). The main metabolites were identified as M700F009 (8–53% of the dose applied), M700F005 (3–9% of the dose applied) and its isomer M700F006 (3–13% of the dose applied), and M700F016 (2–12% of the dose applied) (Tables 7 and 8).

Table 6. Proportions of metabolites identified in rat urine (metabolism study)

Compound	% of administered dose			
	Group DXLA Single high dose (150 mg/kg bw) Aniline label		Group DXLP Single high dose (150 mg/kg bw) Pyrazole label	
	Males (0–168 h)	Females (0–168 h)	Males (0–168 h)	Females (0–168 h)
M700F001	ND	ND	0.20	0.12
M700F002	ND	ND	0.07	0.04
M700F004	0.58	0.25	0.72	0.82
M700F005/M700F024	0.39	ND	0.16	ND
M700F009/M700F028	0.55	ND	0.56	ND
M700F009	ND	1.35	ND	0.84
M700F011	0.04	0.06	0.07	0.05
M700F014	0.94	2.86	1.20	4.82
M700F015	0.82	0.62	0.71	0.97
M700F016	0.25	ND	0.03	0.05
M700F020	0.09	0.06	0.06	0.04
M700F023	0.01	ND	0.04	ND
M700F025	ND	ND	0.01	ND
M700F026/ M700F027	ND	ND	0.06	ND
M700F061	ND	0.23	ND	0.23
Total identified	3.67	5.43	3.89	7.97

From Schopfer & Labib (2009)
ND, not detected/identified

In bile, the main metabolites were in the range of 3.4–22.0% of the applied dose and identified to be M700F004 and its isomer M700F125, M700F009, M700F014 and its isomer M700F123, M700F005, M700F024 and M700F122 (Table 9). In liver, the main metabolites were in the range of 0.1–0.9% of the applied dose and identified as M700F005, M700F006, M700F008 and M700F009 (Table 10). The parent compound was identified at levels up to 3.7% and 0.4% of the dose applied for the low- and high-dose groups, respectively. In kidney, the main metabolite was identified to be M700F008 (0.02–0.11% of the applied dose) (Table 11). The active substance was present at levels up to 0.50% and 0.05% of the applied dose for the low- and high-dose groups, respectively. In fat, two components were detected: the metabolite M700F008 (0.05–0.29% of the applied dose) and unchanged fluxapyroxad (0.35–2.72% of the applied dose) (Table 12). In plasma, the main metabolite was identified to be M700F006 (0.03–0.11% of the applied dose). The parent compound was present at levels up to 0.13% and 0.01% of the applied dose for the low- and high-dose groups, respectively (Table 13).

The main biotransformation steps of fluxapyroxad in rats are hydroxylation at the biphenyl ring system, *N*-demethylation at the pyrazole ring system, loss of a fluorine atom at the biphenyl ring system and conjugation with glucuronic acid or with glutathione derivatives. A further, but negligible, transformation route is cleavage at the amide bond between the pyrazole ring system and the biphenyl ring system.

Table 7. Proportions of metabolites identified in rat faeces (biokinetics study)

Compound	% of administered dose					
	Group B Single low dose (7.5 mg/kg bw)		Group D Single high dose (150 mg/kg bw)		Group C Repeated high dose (14 + 1 × 150 mg/kg bw)	
	Males (0–144 h)	Females (0–120 h)	Males (0–120 h)	Females (0–144 h)	Males (0–120 h)	Females (0–120 h)
M700F005	9.44	8.70	3.53	4.50	2.72	5.22
M700F006	13.30	3.42	6.69	4.45	7.76	6.20
M700F008	0.61	1.69	ND	0.55	0.34	0.34
M700F009	22.24	53.04	7.63	18.21	8.95	10.62
M700F010	4.83	2.34	2.49	2.26	3.23	1.86
M700F016	11.77	3.31	5.43	2.91	9.46	4.17
M700F024	2.90	3.13	2.62	4.90	3.40	7.30
Fluxapyroxad	2.51	3.32	43.81	33.58	30.50	30.70
Total identified	67.60	78.95	72.19	71.36	66.36	66.42

From Schopfer & Labib (2009)
ND, not detected/identified

Table 8. Proportions of metabolites identified in faeces (metabolism study)

Compound	% of administered dose			
	Group DXLA Single high dose (150 mg/kg bw) Aniline label		Group DXLP Single high dose (150 mg/kg bw) Pyrazole label	
	Males (0–168 h)	Females (0–168 h)	Males (0–168 h)	Females (0–168 h)
M700F005	5.49	4.94	6.08	6.82
M700F006	7.13	5.47	9.16	5.88
M700F008	0.78	0.90	1.09	1.12
M700F009	11.37	19.15	14.12	22.50
M700F010	3.14	2.41	3.91	2.43
M700F016	6.89	1.98	8.72	2.24
M700F024	2.22	5.01	2.58	4.54
Fluxapyroxad	30.24	23.41	18.58	26.27
Total identified	67.26	63.28	64.23	71.79

From Schopfer & Labib (2009)

Combination of these reactions led to the observed large number (about 51) of metabolites (see Appendix 1).

The proposed metabolic pathway based on all the rat metabolism studies is shown in Figure 2.

Table 9. Proportions of metabolites identified in bile

Compound	% of administered dose			
	Group R Single low dose (7.5 mg/kg bw) Aniline label		Group S Single high dose (150 mg/kg bw) Aniline label	
	Males (0–72 h)	Females (0–72 h)	Males (0–60 h)	Females (0–72 h)
M700F004	13.99	10.77	21.21	19.46
M700F005/M700F024	9.83	4.23	3.36	4.58
M700F006	0.97	0.16	0.18	0.31
M700F009/M700F125	9.85	13.23	4.18	7.25
M700F014/M700F122	5.41	21.97	9.64	10.90
M700F015/M700F123	3.27	3.91	6.66	10.12
M700F032	0.37	0.03	0.11	0.25
M700F042	0.73	1.26	0.32	0.26
M700F113	1.26	1.25	2.20	8.62
M700F115/M700F116	0.61	0.33	0.75	0.71
M700F117	2.20	1.59	3.80	4.32
M700F118	3.14	0.85	3.01	1.10
M700F120/M700F121	1.73	0.14	1.98	0.95
M700F124	0.24	0.06	ND	0.31
Total identified	53.60	59.79	57.40	69.13

From Schopfer & Labib (2009)
ND, not detected/identified

Table 10. Proportions of metabolites identified in rat liver analysed at T$_{max}$ of plasma level (1 or 16 hours)

Compound	% of administered dose			
	Group V Single low dose (7.5 mg/kg bw) Pyrazole label T_{max} = 1 h		Group W Single high dose (150 mg/kg bw) Pyrazole label T_{max} = 16 h	
	Males	Females	Males	Females
M700F005	0.32	0.54	0.18	0.10
M700F006	0.41	0.10	0.18	0.23
M700F008	0.92	0.85	0.17	0.45
M700F009	0.09	0.07	0.12	0.17
M700F010/M700F103/M700F104	ND	ND	0.02	0.04
M700F016	ND	ND	0.04	0.01
M700F024	0.12	0.03	0.06	0.06
M700F025	ND	ND	0.02	ND
M700F026/M700F102	ND	ND	0.02	ND
M700F105	ND	ND	0.01	ND
Fluxapyroxad	3.03	3.66	0.16	0.36
Total identified	4.89	5.25	0.97	1.42

From Schopfer & Labib (2009)
ND, not detected/identified

Table 11. Proportions of metabolites identified in rat kidney analysed at T_{max} of plasma level (1 or 16 hours)

Compound	% of administered dose			
	Group V Single low dose (7.5 mg/kg bw) Pyrazole label T_{max} = 1 h		Group W Single high dose (150 mg/kg bw) Pyrazole label T_{max} = 16 h	
	Males	Females	Males	Females
M700F005	0.02	0.01	0.01	0.01
M700F006	0.02	0.01	0.01	0.01
M700F008	0.09	0.11	0.02	0.05
M700F009	ND	0.01	0.01	0.01
M700F010	ND	ND	0.00	0.00
M700F050	ND	ND	0.00	0.00
M700F063	ND	ND	ND	0.00
Fluxapyroxad	0.29	0.50	0.03	0.05
Total identified	0.43	0.64	0.08	0.14

From Schopfer & Labib (2009)
ND, not detected/identified

Table 12. Proportions of metabolites identified in rat fat analysed at T_{max} of plasma level (1 or 16 hours)

Compound	% of administered dose			
	Group V Single low dose (7.5 mg/kg bw) Pyrazole label T_{max} = 1 h		Group W Single high dose (150 mg/kg bw) Pyrazole label T_{max} = 16 h	
	Males	Females	Males	Females
M700F008	0.06	0.29	0.04	0.25
Fluxapyroxad	0.41	2.72	0.35	1.55
Total identified	0.47	3.01	0.39	1.81

From Schopfer & Labib (2009)

Table 13. Proportions of metabolites identified in rat plasma analysed at T_{max} of plasma level (1 or 16 hours)

Compound	% of administered dose			
	Group V Single low dose (7.5 mg/kg bw) Pyrazole label T_{max} = 1 h		Group W Single high dose (150 mg/kg bw) Pyrazole label T_{max} = 16 h	
	Males	Females	Males	Females
M700F004/M700F012	0.00	0.00	0.00	0.01
M700F005	0.01	0.00	0.00	0.00
M700F006	0.11	0.05	0.03	0.09
M700F008	0.01	0.02	0.00	0.01

Compound	% of administered dose			
	Group V Single low dose (7.5 mg/kg bw) Pyrazole label T_{max} = 1 h		Group W Single high dose (150 mg/kg bw) Pyrazole label T_{max} = 16 h	
M700F009/M700F013/M700F107	0.00	0.00	0.00	0.01
M700F010	0.01	ND	0.00	0.01
M700F011	ND	ND	0.00	0.00
M700F014	ND	ND	0.00	0.02
M700F015	ND	0.00	0.00	0.00
Fluxapyroxad	0.04	0.13	0.00	0.01
Total identified	0.19	0.22	0.04	0.16

From Schopfer & Labib (2009)
ND, not detected/identified

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

The results of acute toxicity studies with fluxapyroxad administered orally, dermally or by inhalation are summarized in Table 14. All the studies were certified as complying with GLP.

Table 14. Acute toxicity of fluxapyroxad

Species	Strain	Sex	Route	Purity (%)	Result	Reference
Rat	Sprague-Dawley Rj:SD	Female	Oral	99.4	LD_{50} > 2000 mg/kg bw	Sire (2008a)
Rat	Sprague-Dawley Rj:SD	Male and female	Dermal	99.4	LD_{50} > 2000 mg/kg bw	Sire (2008b)
Rat	Wistar	Male and female	Inhalation	98.9	LC_{50} > 5.1 mg/l	Ma-Hock & Landsiedel (2008)

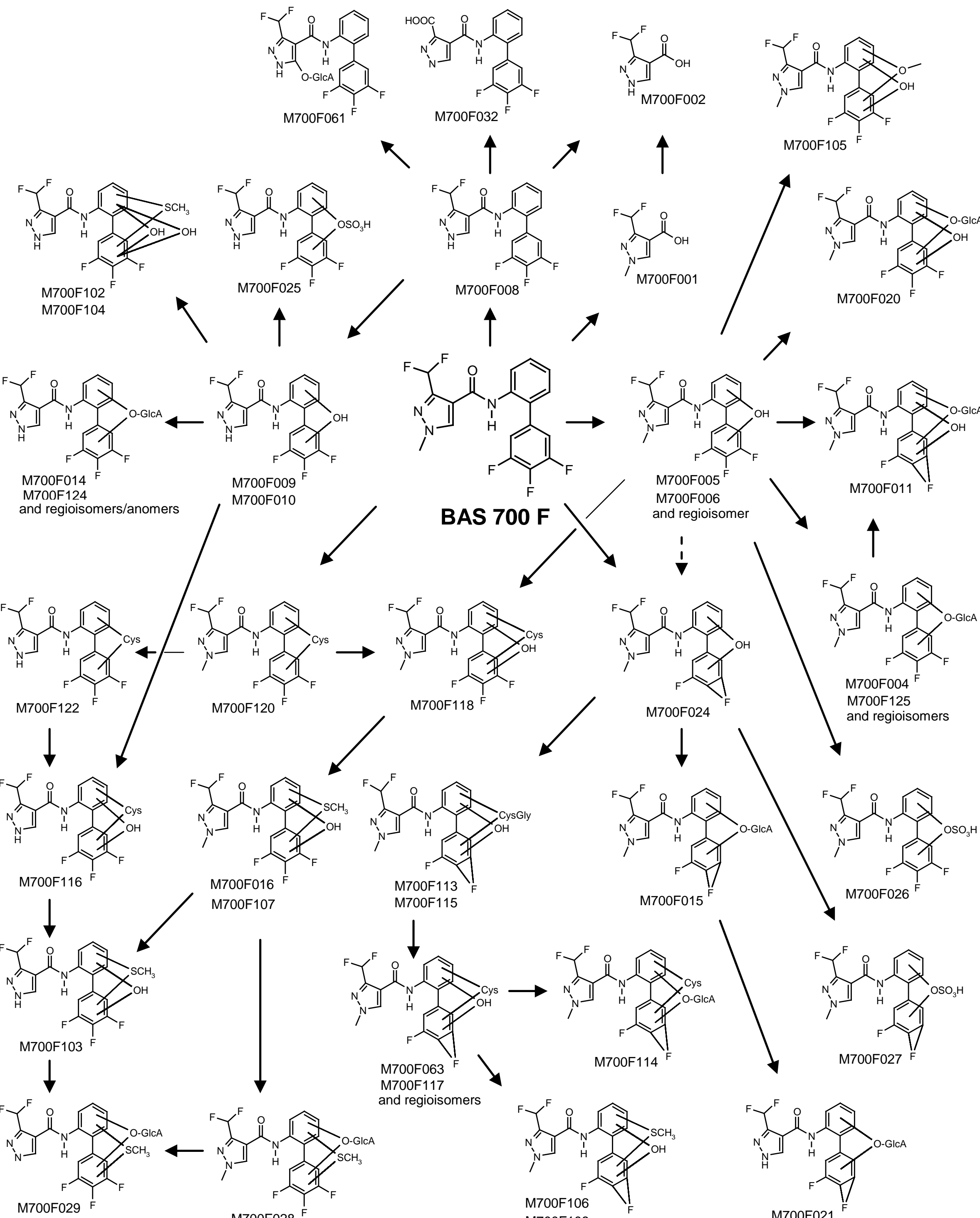
LC_{50}, median lethal concentration; LD_{50}, median lethal dose

Two groups of three young adult female Sprague-Dawley Rj:SD rats were treated once orally, by gavage, with an aqueous suspension of fluxapyroxad at a dose level of 2000 mg/kg bw. The animals were observed for 14 days post-treatment and then subjected to necropsy and postmortem examination.

There were no deaths, and there were no macroscopic findings at necropsy in any animal. Hypoactivity and piloerection were noted in one animal 2–4 hours after dosing. All animals gained weight throughout the study period. Based on these results, the acute oral median lethal dose (LD_{50}) was estimated to be greater than 2000 mg/kg bw (Sire, 2008a).

A group of five male and five young adult female Sprague-Dawley Rj:SD rats was treated with fluxapyroxad once for 24 hours by topical, semi-occluded application to a clipped area of intact dorsal skin (approximately 10% of the body surface area) at a dose level of 2000 mg/kg bw (moistened with 2 ml purified water). The animals were observed for 14 days post-treatment and then subjected to necropsy and postmortem examination.

Figure 2. Proposed metabolic pathway of fluxapyroxad (BAS 700 F) in rat



Remark: CysGlyc = cysteineglycine, Cys = cysteine; GlcA = glucuronic acid

From Schopfer & Labib (2009)

No deaths or systemic clinical signs of an adverse reaction to treatment occurred. Erythema was noted in two animals of each sex; however, there were no macroscopic findings at necropsy in any animal. All animals gained weight throughout the study period. Based on these results, the acute dermal LD_{50} was estimated to be greater than 2000 mg/kg bw (Sire, 2008b).

A group of five male and five female Wistar rats was exposed once for 4 hours by nose-only, flow-past inhalation to a dust aerosol of fluxapyroxad at an analytically determined mean concentration of 5.1 mg/l, with mass median aerodynamic diameter (MMAD) ± geometric standard deviation (GSD) of 3.3 µm ± 2.1 µm / 3.4 µm ± 2.2 µm. The animals were observed for 14 days post-treatment, during which time clinical signs and body weights were recorded, and then subjected to necropsy and postmortem examination.

No deaths occurred during the exposure or observation periods. Clinical signs of toxicity included increased respiration, abdominal respiration, piloerection and squatting posture beginning at hour 2 during exposure. Clinical signs persisted for up to 6 days. All animals gained weight throughout the study period. There were no macroscopic findings at necropsy in any animal. Based on these results, the acute median lethal concentration (4-hour LC_{50}) was estimated to be greater than 5.1 mg/l air (Ma-Hock & Landseidel, 2008).

(b) Ocular irritation

In a primary eye irritation study, approximately 0.1 ml of fluxapyroxad (purity 99.7%) was instilled into the right conjunctival sac of one male and two female New Zealand White rabbits, with irrigation after 24 hours.

No corneal or iridial lesions occurred in the animals at any observation interval. All three animals showed grade 1 conjunctival redness at 1 hour after instillation. All animals were free of ocular reactions 24, 48 and 72 hours after instillation. Fluxapyroxad is not irritating to rabbit eyes (Bauer & Landsiedel, 2008).

(c) Dermal irritation

In a primary skin irritation study, 0.5 g of fluxapyroxad (purity 99.7%) moistened with water was applied to the shorn skin of one female and two male young adult New Zealand White rabbits. The treated skin area was 2.5 cm × 2.54 cm, and exposure lasted for 4 hours.

Slight erythema was observed in all rabbits at removal of dressing and at 1 hour. Irritation persisted to 24 hours in one rabbit; all dermal irritation was resolved by 72 hours. Fluxapyroxad was slightly irritating to rabbit skin (Remmele & Hellwig, 2006).

(d) Dermal sensitization

The skin sensitization potential of fluxapyroxad (purity 99.7%) was investigated in the maximization test in female Dunkin-Hartley guinea-pigs (20 test and 10 negative control). A positive control study with α-hexylcinnaldehyde was conducted within 6 months of the study, which demonstrated the ability of the laboratory to elicit a positive response. Concentrations of 5%, 60% and 25% fluxapyroxad were used for intradermal induction, topical induction and challenge, respectively. Skin reactions to the challenge applications were evaluated 24 and 48 hours after patch removal. The concentrations of fluxapyroxad applied were determined in preliminary irritation studies.

Following challenge, 2 of the 20 test animals had a grade 1 reaction at 24 hours, and 1 had a grade 1 reaction at 48 hours. One of the 10 negative control animals showed a reaction to the challenge application at 24 hours (grade 2) and 48 hours (grade 1) after patch removal. Fluxapyroxad was not sensitizing to skin in the maximization test in guinea-pigs (Gamer & Landsiedel, 2008).

The above ocular and dermal irritation and skin sensitization studies are summarized in Table 15.

Table 15. Irritation and skin sensitization potential of fluxapyroxad

Species	Strain	Sex	End-point (method)	Purity (%)	Result	Reference
Rabbit	New Zealand White	Male and female	Skin irritation	99.7	Slightly irritating	Remmele & Hellwig (2006)
Rabbit	New Zealand White	Male and female	Eye irritation	99.7	Not irritating	Bauer & Landsiedel (2008)
Guinea-pig	Dunkin-Hartley	Female	Skin sensitization (Magnusson & Kligman)	99.7	Not sensitizing	Gamer & Landsiedel (2008)

2.2 *Short-term studies of toxicity*

(a) Oral administration

Mice

In a 28-day range-finding study, groups of five C57BL/6 J Rj mice of each sex per dose received fluxapyroxad (purity 99.1%) in the diet at a concentration of 0, 500, 2500 or 7000 ppm (equal to 0, 112, 552 and 1452 mg/kg bw per day for males and 0, 150, 746 and 2100 mg/kg bw per day for females, respectively) for at least 28 days. Clinical signs were recorded daily, and a detailed physical examination was performed weekly. Body weights and feed consumption were recorded weekly, and haematology and plasma clinical chemistry were performed after 4 weeks of treatment, prior to necropsy. All animals were subjected to necropsy, postmortem examination, organ weight recording and tissue preservation. The liver and thyroid of all animals were processed and examined by light microscopy. Data were analysed statistically where appropriate.

There were no deaths and no adverse clinical signs at any dose level. Body weight gain and feed consumption were decreased at the high dose in males only (Table 16). Haematological changes occurred in males only at 7000 ppm (Table 17). These changes included reduced haemoglobin levels, a lower haematocrit and decreased (58%) white blood cell count. This decrease in white blood cell count was accompanied by a non-significant decrease in the absolute and relative neutrophil and lymphocyte cell counts, as well as an increase in the absolute and relative monocyte cell counts. Clinical chemistry changes were also observed in 7000 ppm males and included increased plasma alkaline phosphatase (ALP) in conjunction with increased blood urea; changes in urea and total protein in females were not considered toxicologically significant due to the magnitude of change and/or lack of a dose–response relationship (Table 18).

Table 16. Mean body weight and body weight gain of mice administered fluxapyroxad for at least 28 days

	Mean body weight / body weight gain ± SD (g)							
	Males (n = 5/dose)				Females (n = 5/dose)			
	0 ppm	500 ppm	2500 ppm	7000 ppm	0 ppm	500 ppm	2500 ppm	7000 ppm
Body weight								
- day 0	22.0 ± 0.7	23.0 ± 1.2	22.0 ± 0.0	22.0 ± 0.7	18.6 ± 0.5	18.2 ± 0.4	18.6 ± 0.5	18.2 ± 0.4
- day 28	25.4 ± 0.5	25.9 ± 1.1	25.9 ± 0.6	24.5 ± 0.4 (↓3.6%)	20.1 ± 0.8	20.7 ± 0.5	21.0 ± 0.7	19.9 ± 1.0
Overall body weight gain	3.4 ± 0.4	2.9 ± 2.2	3.9 ± 0.6	2.5 ± 0.7 (↓27.2%)	1.5 ± 1.1	2.5 ± 0.6	2.4 ± 0.6	1.7 ± 1.3

From Kamp et al. (2009a)
SD, standard deviation

Table 17. Selected haematology findings in mice administered fluxapyroxad for at least 28 days

	Group mean values ± SD							
	Males (n = 4/dose at 0 ppm, n = 5/dose at ≥ 500 ppm)				Females (n = 5/dose)			
	0 ppm	500 ppm	2500 ppm	7000 ppm	0 ppm	500 ppm	2500 ppm	7000 ppm
White blood cells (10^9/l)	3.90 ± 1.45	3.97 ± 0.37	3.74 ± 0.94	1.64* ± 0.63 (−58%)	1.17 ± 0.26	1.58 ± 1.08	1.52 ± 0.269	2.62 ± 1.93
Haemoglobin (mmol/l)	9.7 ± 0.2	9.5 ± 0.5	9.5 ± 0.5	9.0* ± 0.3 (−7%)	9.3 ± 0.2	9.3 ± 0.3	9.3 ± 0.2	9.1 ± 0.5
Haematocrit (l/l)	0.456 ± 0.007	0.449 ± 0.022	0.450 ± 0.013	0.420* ± 0.013 (−8%)	0.439 ± 0.015	0.438 ± 0.022	0.432 ± 0.014	0.422 ± 0.020
Red blood cells (10^{12}/l)	10.17 ± 0.13	10.05 ± 0.48	10.06 ± 0.25	9.50 ± 0.34	9.70 ± 0.85	9.79 ± 0.41	9.70 ± 0.29	9.58 ± 0.48
Mean corpuscular volume (fl)	44.9 ± 0.3	44.7 ± 0.2	44.7 ± 0.4	44.2 ± 0.5	45.2 ± 0.2	44.7 ± 0.5	44.6* ± 0.5 (−1%)	44.0** ± 0.2 (−3%)
Neutrophils, relative (%)	25.2 ± 5.8	30.8 ± 3.0	29.7 ± 6.3	23.4 ± 10.9	43.8 ± 20.7	38.5 ± 15.7	26.3 ± 10.7	23.6 ± 8.9
Neutrophils, absolute (10^9/l)	0.48 ± 0.17	0.61 ± 0.15	0.55 ± 0.15	0.17 ± 0.05	0.26 ± 0.17	0.34 ± 0.29	0.19 ± 0.04	0.35 ± 0.34
Lymphocytes, relative (%)	84.7 ± 3.3	81.2 ± 3.5	81.8 ± 3.5	77.3 ± 12.9	72.8 ± 9.4	75.9 ± 8.8	77.7 ± 8.4	83.0 ± 5.4
Lymphocytes, absolute (10^9/l)	3.32 ± 1.31	3.23 ± 0.40	3.07 ± 0.32	1.30 ± 0.65	0.85 ± 0.17	1.19 ± 0.84	1.20 ± 0.35	2.15 ± 1.54
Monocytes, relative (%)	0.4 ± 0.1	0.4 ± 0.2	0.8 ± 0.3	6.5 ± 7.1	0.8 ± 0.4	1.1 ± 0.7	3.9 ± 3.6	2.4 ± 1.5
Monocytes, absolute (10^9/l)	0.02 ± 0.01	0.01 ± 0.01	0.03 ± 0.03	0.11 ± 0.09	0.01 ± 0.01	0.02 ± 0.02	0.05 ± 0.05	0.07 ± 0.06

From Kamp et al. (2009a)
* $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two-sided)

Table 18. Selected clinical chemistry findings in mice administered fluxapyroxad for at least 28 days

	Group mean values ± SD							
	Males (n = 5/dose)				Females (n = 5/dose)			
	0 ppm	500 ppm	2500 ppm	7000 ppm	0 ppm	500 ppm	2500 ppm	7000 ppm
ALP (µkat/l)	2.27 ± 0.13	2.05* ± 0.10	2.14 ± 0.11	2.90* ± 0.53 (+28%)	3.77 ± 0.56	3.33 ± 0.27	3.31 ± 0.52	4.01 ± 0.37
Na^+ (mmol/l)	149.1 ± 0.07	150.1 ± 1.9	150.0 ± 1.0	152.5** ± 1.2 (+2%)	149.7 ± 1.5	150.0 ± 1.4	151.4 ± 1.2	149.9 ± 2.8
K^+ (nmol/l)	6.86 ± 0.76	6.88 ± 0.59	6.15 ± 0.29	5.77* ± 0.36 (−16%)	5.58 ± 0.52	5.99 ± 0.40	5.54 ± 0.26	5.70 ± 0.51

Table 18 (continued)

	Group mean values ± SD							
	Males (*n* = 5/dose)				Females (*n* = 5/dose)			
	0 ppm	500 ppm	2500 ppm	7000 ppm	0 ppm	500 ppm	2500 ppm	7000 ppm
Urea (mmol/l)	12.56 ± 0.67	12.60 ± 0.72	11.59 ± 1.34	15.42** ± 1.72 (+23%)	14.53 ± 1.39	13.72 ± 1.62	11.72** ± 0.87 (−19%)	12.12* ± 1.01 (−17%)
Protein, total (g/l)	57.51 ± 0.55	55.30** ± 0.72 (−7%)	52.10** ± 1.25 (−9%)	49.92** ± 1.53 (−13%)	55.11 ± 3.09	51.32** ± 1.06 (−7%)	51.39* ± 1.59 (−7%)	46.26** ± 2.54 (−16%)
Albumin (g/l)	37.31 ± 0.49	35.75** ± 0.28 (−4%)	33.75** ± 0.28 (−10%)	32.08** ± 0.97 (−14%)	36.99 ± 1.27	34.66* ± 0.84 (−6%)	34.16** ± 0.90 (−8%)	30.94** ± 1.66 (−16%)
Globulin (g/l)	20.20 ± 0.29	19.55 ± 0.53	18.34** ± 0.45 (−9%)	17.85** ± 0.64 (−12%)	18.12 ± 1.83	16.47* ± 0.35 (−9%)	17.23 ± 0.76	15.32** ± 1.01 (−15%)
Triglycerides (mmol/l)	0.99 ± 0.22	0.85 ± 0.30	0.63* ± 0.06 (−36%)	0.34** ± 0.06 (−66%)	0.36 ± 0.14	0.31 ± 0.12	0.28 ± 0.06	0.19 ± 0.03
Cholesterol (mmol/l)	2.63 ± 0.11	2.22** ± 0.18 (−16%)	1.48** ± 0.17 (−44%)	0.87** ± 0.10 (−67%)	1.65 ± 0.07	1.37* ± 0.20 (−17%)	1.07** ± 0.18 (−35%)	0.99** ± 0.11 (−40%)

From Kamp et al. (2009a)
ALP, alkaline phosphatase; * $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)

The no-observed-adverse-effect level (NOAEL) in males was 2500 ppm (552 mg/kg bw per day), based on haematological changes observed at 7000 ppm (equal to 1452 mg/kg bw per day). The NOAEL in females was 7000 ppm (equal to 2100 mg/kg bw per day), the highest dose tested (Kamp et al., 2009a).

In a 90-day oral toxicity study, groups of 10 C57BL/6 J Rj mice of each sex per dose received fluxapyroxad (purity 99.6%) in the diet at a concentration of 0, 100, 400, 2000 or 6000 ppm (equal to 0, 21, 77, 390 and 1136 mg/kg bw per day for males and 0, 32, 128, 610 and 1657 mg/kg bw per day for females, respectively) for 13 weeks. Clinical signs were recorded at least daily, and a detailed physical examination was performed weekly. Body weights and feed consumption were recorded weekly, and haematology and plasma clinical chemistry evaluations were performed in week 13, prior to necropsy. All animals were subjected to necropsy, postmortem examination, organ weight recording and tissue preservation. Major organs and tissues from all animals were processed and examined by light microscopy. In order to assess the effects on the liver, an immunohistochemical determination of cell proliferation was conducted with liver samples of male animals of the top-dose and control groups. The slides were dewaxed, rehydrated and covered with phosphate-buffered saline. They were then incubated consecutively with a primary antibody (mouse anti-human proliferating cell nuclear antigen), a secondary antibody (rabbit anti-mouse), a streptavidin-biotin enzyme label and a chromogen substrate solution (Fast Red). Sections were counterstained with Mayer’s haematoxylin only. Data were analysed statistically where appropriate.

There were no deaths and no treatment-related clinical signs at any dose level. Significant decreases in body weight (12.6%) and body weight gain (32.5%) were observed in top-dose males, and feed consumption was decreased at the high dose in both sexes (Table 19).

Table 19. Group mean body weight, body weight gain and feed consumption in mice at selected intervals

	Group mean values ± SD									
	Males					Females				
	0 ppm	100 ppm	400 ppm	2000 ppm	6000 ppm	0 ppm	100 ppm	400 ppm	2000 ppm	6000 ppm
Body weight (g)										
- day 0	21.9 ± 0.7	21.6 ± 0.8	21.8 ± 0.8	21.8 ± 0.8	21.5 ± 0.5	17.5 ± 0.5	17.5 ± 0.5	17.4 ± 0.7	17.6 ± 0.7	17.3 ± 0.8
- day 91	34.0 ± 2.3	32.9 ± 2.1	32.6 ± 2.7	33.1 ± 2.0	29.7** ± 0.9	22.8 ± 1.2	23.9 ± 2.2	23.3 ± 1.9	23.2 ± 1.1	22.6 ± 1.1
% change[a]	—	*−3.2*	*−4.3*	*−2.7*	*−12.6*	—	*+4.8*	*+2.2*	*+1.6*	*−1.1*
Overall body weight gain (g)	12.1 ± 2.3	11.4 ± 2.1	10.8 ± 2.7	11.3 ± 2.2	8.2** ± 0.8	5.4 ± 1.1	6.4 ± 2.5	5.9 ± 1.6	5.6 ± 1.4	5.3 ± 1.1
% change[a]	—	*−6.3*	*−11.2*	*−7.1*	*−32.5*	—	*+20.4*	*+10.8*	*+4.5*	*−1.1*
Feed consumption (g/animal)										
- cumulative (days 0–91)	518.0	523.6	487.2	498.4	466.2	618.8	635.6	626.5	597.8	529.2
% change[a]	—	*+1.1*	*−5.9*	*−3.8*	*−10.0*	—	*+2.7*	*+1.2*	*−3.4*	*−14.5*

From Kamp et al. (2009b)
SD, standard deviation; ** $P \leq 0.01$ (Dunnett's test, two-sided)
[a] Compared with control.

The liver was identified as a target organ, as indicated by changes in a number of clinical chemistry parameters down to a dietary concentration of 400 ppm in males (Table 20) as well as statistically significant increases in absolute and relative liver weights at 2000 ppm or higher in both sexes (Table 21). The clinical chemistry changes consisted of increased ALP, alanine aminotransferase (ALT) and urea levels in top-dose males, as well as decreased triglyceride (males only) and albumin, total protein and cholesterol levels in both sexes. An increased incidence of multifocal necrosis was observed in the liver of 5 of 10 males at 6000 ppm (Table 22) and, in conjunction with increased liver enzymes, was considered potentially adverse. There was no effect on liver cell proliferation (Table 23).

Table 20. Group mean values for select plasma chemistry parameters in mice

	Group mean values ± SD									
	Males					Females				
	0 ppm	100 ppm	400 ppm	2000 ppm	6000 ppm	0 ppm	100 ppm	400 ppm	2000 ppm	6000 ppm
ALP (µkat/l)	1.02 ± 0.08	1.03 ± 0.08	1.00 ± 0.11	1.05 ± 0.08	1.21** ± 0.12 (+19%)	2.13 ± 0.27	1.99 ± 0.33	1.99 ± 0.26	2.11 ± 0.26	2.31 ± 0.30
ALT (µkat/l)	1.00 ± 0.12	1.00 ± 0.13	1.11 ± 0.29	1.11 ± 0.19	1.51** ± 0.48 (+51%)	1.77 ± 0.71	1.33 ± 0.44	1.32 ± 0.30	1.60 ± 0.41	1.93 ± 0.54
Urea (mmol/l)	8.09 ± 0.77	8.41 ± 1.14	7.97 ± 1.11	8.18 ± 0.86	9.47** ± 0.84 (+17%)	11.66 ± 2.42	9.42 ± 2.51	10.43 ± 2.27	11.66 ± 3.12	11.83 ± 2.39

Table 20 (continued)

	Group mean values ± SD									
	Males					Females				
	0 ppm	100 ppm	400 ppm	2000 ppm	6000 ppm	0 ppm	100 ppm	400 ppm	2000 ppm	6000 ppm
Protein, total (g/l)	53.79 ± 1.43	53.85 ± 1.12	53.13 ± 2.09	52.62 ± 1.77	50.33** ± 1.78 (−6%)	52.21 ± 2.59	52.74 ± 2.67	52.81 ± 3.24	49.44* ± 2.48 (−5%)	49.23* ± 2.74 (−6%)
Albumin (g/l)	34.00 ± 1.05	33.95 ± 1.29	33.62 ± 1.15	33.93 ± 1.20	31.53** ± 1.21 (−7%)	34.64 ± 1.16	34.72 ± 1.54	34.73 ± 1.99	32.83** ± 1.39 (−5%)	32.30** ± 1.52 (−7%)
Triglycerides (mmol/l)	1.18 ± 0.22	1.00 ± 0.25	0.94* ± 0.18 (−20%)	0.82** ± 0.16 (−31%)	0.49** ± 0.10 (−58%)	0.46 ± 0.19	0.55 ± 0.18	0.49 ± 0.17	0.38 ± 0.16	0.35 ± 0.15
Cholesterol (mmol/l)	2.53 ± 0.20	2.44 ± 0.18	2.21** ± 0.21 (−13%)	1.79** ± 0.20 (−29%)	1.20** ± 0.18 (−53%)	1.85 ± 0.39	1.96 ± 0.34	1.84 ± 0.36	1.41** ± 0.24 (−24%)	1.24** ± 0.16 (−33%)

From Kamp et al. (2009b)

ALP, alkaline phosphatase; ALT, alanine aminotransferase; SD, standard deviation; * $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)

Table 21. Group mean body weights and selected absolute and relative organ weights at 13 weeks in mice

	Dietary concentration (ppm)	Group mean values ± SD							
		Males				Females			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
Terminal weight (g)	0	30.02 ± 2.24	—	—	—	18.47 ± 1.21	—	—	—
	100	29.04 ± 2.31	−3.3	—	—	19.99 ± 2.21	+8.2	—	—
	400	28.84 ± 2.65	−3.9	—	—	19.67 ± 2.21	+6.5	—	—
	2000	28.94 ± 2.187	−3.6	—	—	19.27 ± 0.82	+4.3	—	—
	6000	25.81** ± 1.04	−14.0	—	—	18.78 ± 1.24	+1.7	—	—
Liver (g)	0	1.16 ± 0.073	—	3.871 ± 0.220	—	0.822 ± 0.092	—	4.44 ± 0.287	—
	100	1.167 ± 0.062	+0.6	4.028 ± 0.171	+4.1	0.91 ± 0.11	+10.7	4.56 ± 0.293	+2.6
	400	1.187 ± 0.084	+2.3	4.12 ± 0.27	+6.5	0.888 ± 0.11	+8.0	4.52 ± 0.301	+1.7
	2000	1.316** ± 0.066	+13.4	4.565** ± 0.33	+17.9	0.961** ± 0.098	+16.9	4.99** ± 0.48	+12.4
	6000	1.479** ± 0.083	+27.5	5.73 ** ± 0.29	+48.0	1.068** ± 0.12	+29.9	5.67** ± 0.32	+27.8
Spleen (g)	0	0.066 ± 0.013	—	0.224 ± 0.044	—	0.054 ± 0.012	—	0.287 ± 0.053	—
	100	0.063 ±	−4.5	0.22 ±	−1.0	0.06 ±	+11.1	0.296 ±	+2.5

Dietary concentration (ppm)	Group mean values ± SD							
	Males				Females			
	Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
	0.008		0.038		0.015		0.044	
400	0.06 ± 0.007	−9.1	0.21 ± 0.027	−5.2	0.059 ± 0.010	+9.3	0.305 ± 0.044	+3.3
2000	0.057 ± 0.007	−13.6	0.195 ± 0.016	−10.6	0.061 ± 0.014	+13.0	0.305 ± 0.052	+8.5
6000	0.053* ± 0.005	−19.7	0.203 ± 0.019	−6.8	0.053 ± 0.007	−1.9	0.288 ± 0.024	+3.0

From Kamp et al. (2009b)

SD, standard deviation; * $P < 0.05$; ** $P < 0.01$

[a] Compared with control. Values may not calculate exactly due to rounding.

[b] Percentage of body weight.

Table 22. Incidence of selected histopathological lesions in mice

	Males ($n = 10$ per group)					Females ($n = 10$ per group)				
	0 ppm	100 ppm	400 ppm	2000 ppm	6000 ppm	0 ppm	100 ppm	400 ppm	2000 ppm	6000 ppm
Glandular stomach										
No. examined	*10*	—	—	—	*10*	*10*	*1*	*1*	*2*	*10*
Erosion/ulcer	—	—	—	—	—	4	1	1	2	2
Liver										
No. examined	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*
Fatty change, diffuse	10 [1.8][a]	10 [1.7]	10 [2.2]	10 [2.8]	10 [2.6]	10 [2.4]	10 [2.6]	10 [2.5]	10 [2.4]	10 [2.1]
Necrosis, (multi)focal	—	—	2 [1.0]	1 [1.0]	5 [1.6]	—	—	2 [2.5]	—	1 [1.0]
Infiltration, lymphoid, (multi)focal	5 [1.6]	4 [1.0]	7 [1.0]	9 [1.0]	7 [1.3]	3 [1.0]	7 [1.0]	5 [1.0]	5 [1.0]	6 [1.0]
Pigment storage, diffuse	—	—	—	—	1 [1.0]	—	—	—	—	—
Spleen										
No. examined	*10*	—	—	—	*10*	*10*	—	—	—	*10*
Haematopoiesis, extramedullar	4	—	—	—	5	5	—	—	—	8
Pigment storage	—	—	—	—	1	9	—	—	—	10
Thymus										
No. examined	*10*	—	—	—	*10*	*10*	—	—	—	*10*
Starry sky cells, increase	—	—	—	—	—	9	—	—	—	8
Atrophy, diffuse	—	—	—	—	—	1	—	—	—	—
Cyst(s)	1	—	—	—	2	2	—	—	—	1

From Kamp et al. (2009b)

[a] [ ] mean severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked (Grade 4) and massive/severe (Grade 5). The mean severity is the sum of the gradings divided by the incidence of the respective finding.

Table 23. Number of proliferating cell nuclear antigen–positive hepatocytes in control and high-dose male mice

	Number of hepatocytes			
	Zone 1	Zone 2	Zone 3	Zones 1–3
Control				
- total	3	59	11	73
- mean	0.3	5.9	1.1	7.3
- SD	0.48	5.78	1.29	6.38
6000 ppm				
- total	9	71	13	93
- mean	0.9	7.1	1.3	9.3
- SD	1.29	3.78	1.83	4.79

From Kamp et al. (2009b)
SD, standard deviation

The NOAEL in males was 2000 ppm (equal to 390 mg/kg bw per day), based on decreased body weight and body weight gain and multifocal necrosis in the liver at 6000 ppm (equal to 1136 mg/kg bw per day). The NOAEL in females was 6000 ppm (equal to 1657 mg/kg bw per day), the highest dose tested (Kamp et al., 2009b).

Rats

In a 4-week range-finding study, groups of five Wistar rats of each sex per dose received fluxapyroxad (purity 99.81%) in the diet at a concentration of 0, 100, 500, 2000 or 6000 ppm (equal to 0, 9.0, 43.7, 176 and 530 mg/kg bw per day for males and 0, 9.4, 47.8, 183 and 531 mg/kg bw per day for females, respectively). Clinical signs were recorded at least daily, a detailed physical examination was performed weekly and a functional observational battery was performed in week 4. Body weights and feed consumption were recorded weekly, and haematology, plasma clinical chemistry and urine analysis were performed in week 4, prior to necropsy. All animals were subjected to necropsy, postmortem examination, organ weight recording and tissue preservation. Major organs and tissues from the control and high-dose groups and liver, kidneys and gross lesions from all animals were processed and examined by light microscopy. Data were analysed statistically where appropriate.

Decreased feed consumption was noted in top-dose females (6000 ppm) only, but it was not accompanied by corresponding body weight effects and so was not considered adverse. No clinical signs of toxicity were reported, and there were no deaths. Absolute and relative liver weights were increased at 500 ppm and above, and histopathological changes (increased incidence and severity of centrilobular hepatocyte hypertrophy in males at 500 ppm and above and in females at 2000 ppm and above) were observed (Table 24). These changes were considered adaptive and not adverse.

The clinical chemistry changes consisted of increased serum gamma-glutamyl transferase (GGT) at 6000 ppm in both sexes (which was considered equivocal), increased total protein and globulin levels at 500 ppm and above in males and at 2000 ppm and above in females (which were not considered adverse) and increased triglyceride and cholesterol levels (which were considered potentially adverse); triglyceride levels were elevated in 6000 ppm females only, whereas a dose-dependent increase in cholesterol was observed in males at 500 ppm and above and in females at 2000 ppm and above. Changes in electrolyte levels (calcium, chloride and inorganic phosphate ions) were observed in females and/or males and were not considered adverse (Table 25). Haematological parameters, including prothrombin time, were evaluated for both sexes (Table 26). No effects were observed in males. However, shorter prothrombin times were observed in females at 2000 ppm and higher. This was considered potentially adverse due to the possibility of increased blood coagulation. There were no effects on urine analysis parameters.

Table 24. Incidence and severity[a] of selected macropathological and histopathological lesions in rats administered fluxapyroxad for at least 28 days

	Males (*n* = 5/group)					Females (*n* = 5/group)				
	0 ppm	100 ppm	500 ppm	2000 ppm	6000 ppm	0 ppm	100 ppm	500 ppm	2000 ppm	6000 ppm
Macropathology										
Liver										
No. examined	5	5	5	5	5	5	5	5	5	5
Enlarged	0	0	0	2	5	0	0	0	0	2
Histopathology										
Liver										
No. examined	5	5	5	5	5	5	5	5	5	5
Hypertrophy, centrilobular	0	0	5	5	5	0	0	0	5	5
			[1.4]	[4.0]	[4.2]				[2.4]	[4.0]
Thyroid										
No. examined	5	5	5	5	5	5	5	5	5	5
Hypertrophy/hyperplasia, follicular	1	1	4	5	4	0	0	1	0	1
	[1.0]	[1.0]	[1.3]	[1.4]	[1.5]			[1.0]		[2.0]
Altered colloid	0	0	1	2	2	0	0	0	0	0
			[1.0]	[1.3]	[1.5]					
Femur										
No. examined	5	5	5	5	5	5	5	5	5	5
Perl's Prussian Blue stain	0	0	0	2	5	0	0	0	2	5
				[1.0]	[2.6]				[1.0]	[2.8]

From Kamp et al. (2009c)

[a] [ ] mean severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked (Grade 4) and massive/severe (Grade 5). The mean severity is the sum of the gradings divided by the incidence.

In the thyroid, changes in thyroid stimulating hormone (TSH) levels (Table 25) and hypertrophy of thyroid follicular cells and altered colloid (Table 24) were observed in males at and above 500 ppm. These changes were considered adverse.

Deposition of Perl's Prussian Blue stainable material (probably Fe^{3+}) was observed in the femur of both sexes at 2000 ppm and higher (Table 24). Although there were no other microscopic changes or structural changes of the bone architecture, this effect was considered potentially adverse.

The NOAEL in males was 100 ppm (equal to 9.0 mg/kg bw per day), based on changes in thyroid hormones and thyroid follicular hypertrophy and hyperplasia and clinical chemistry changes seen at 500 ppm (equal to 43.7 mg/kg bw per day). The NOAEL in females was 500 ppm (equal to 47.8 mg/kg bw per day), based on decreased prothrombin time and clinical chemistry changes seen at 2000 ppm (equal to 183 mg/kg bw per day) (Kamp et al., 2009c).

Table 25. Selected clinical chemistry findings in rats administered fluxapyroxad for at least 28 days

	Group mean values ± SD									
	Males (n = 5/group)					Females (n = 5/group)				
	0 ppm	100 ppm	500 ppm	2000 ppm	6000 ppm	0 ppm	100 ppm	500 ppm	2000 ppm	6000 ppm
GGT (µkat/l)	0 ± 0	0 ± 0	1 ± 2	5 ± 5	223** ± 109	1 ± 3	0 ± 0	0 ± 0	1 ± 1	96** ± 48
ALT (µkat/l)	0.50 ± 0.06	0.48 ± 0.05	0.48 ± 0.08	0.48 ± 0.11	0.57 ± 0.06	0.43 ± 0.06	0.35 ± 0.05	0.36 ± 0.09	0.31 ± 0.06	0.29 ± 0.04
ALP (µkat/l)	2.49 ± 0.41	2.14 ± 0.40	2.43 ± 0.31	1.85 ± 0.20	2.14 ± 0.44	1.35 ± 0.35	1.24 ± 0.37	1.07 ± 0.23	0.97 ± 0.11	0.94 ± 0.12
AST (µkat/l)	2.03 ± 0.52	1.82 ± 0.09	1.55 ± 0.24	1.46 ± 0.40	1.75 ± 0.49	1.77 ± 0.22	1.66 ± 0.22	1.56 ± 0.15	1.40 ± 0.39	1.14** ± 0.15 (−36%)
K^+ (mmol/l)	4.35 ± 0.26	4.77* ± 0.16 (+9.7%)	4.70* ± 0.16 (+8.0%)	4.41 ± 0.23	4.47 ± 0.10	4.28 ± 0.23	4.20 ± 0.12	4.10 ± 0.28	4.21 ± 0.28	4.48 ± 0.17
Cl^- (mmol/l)	103 ± 1.4	102.6 ± 0.07	102.8 ± 1.3	101.3 ± 1.8	99.1** ± 2.3 (−3.8%)	102.9 ± 1.0	104 ± 1.5	104.7* ± 1.3 (+1.7%)	103.7 ± 0.9	101.6 ± 1.5
PO_4^{3-}, inorganic (mmol/l)	2.34 ± 0.18	2.39 ± 0.17	2.39 ± 0.04	2.55 ± 0.21	2.69** ± 0.07 (+15%)	2.35 ± 0.20	2.07 ± 0.16	2.12 ± 0.27	2.22 ± 0.09	2.34 ± 0.26
Ca^{2+} (mmol/l)	2.54 ± 0.06	2.60 ± 0.03	2.63* ± 0.04 (+3.5%)	2.65* ± 0.05 (+4.3%)	2.74** ± 0.09 (+7.9%)	2.59 ± 0.08	2.55 ± 0.08	2.53 ± 0.05	2.62 ± 0.05	2.73* ± 0.08 (+5.4%)
Protein, total (g/l)	64.93 ± 0.83	67.03 ± 1.74	68.43** ± 2.12 (+5.3%)	69.68** ± 1.28 (+7.3%)	72.03* ± 4.38 (+11%)	67.49 ± 1.79	65.86 ± 2.75	66.97 ± 3.01	71.86** ± 1.72 (+6.5%)	74.63** ± 2.51 (+11%)
Globulin (g/l)	26.59 ± 0.94	27.44 ± 1.59	28.90* ± 0.94 (+8.7%)	31.70** ± 3.38 (+19%)	32.56** ± 2.23 (+22%)	26.11 ± 0.74	25.63 ± 1.05	26.3 ± 1.38	29.82** ± 0.76 (+14%)	31.07** ± 1.04 (+19%)
Triglycerides (mmol/l)	0.52 ± 0.18	0.47 ± 0.12	0.57 ± 0.16	0.83 ± 1.04	2.93 ± 4.80	0.27 ± 0.05	0.26 ± 0.05	0.22 ± 0.04	0.36 ± 0.12	0.42* ± 0.11 (+56%)
Cholesterol (mmol/l)	1.93 ± 0.17	2.06 ± 0.11	2.48* ± 0.29 (+28%)	4.37** ± 2.94 (+120%)	6.44** ± 3.73 (+230%)	1.38 ± 0.46	1.49 ± 0.31	1.67 ± 0.23	2.72* ± 0.55 (+97%)	3.91** ± 0.44 (+180%)
Mg^{2+} (mmol/l)	1.00 ± 0.02	0.95* ± 0.03 (−5.0%)	0.99 ± 0.03	1.01 ± 0.04	1.06 ± 0.06	1.07 ± 0.02	1.06 ± 0.03	1.03 ± 0.03	1.09 ± 0.08	1.06 ± 0.03
T_3 (nmol/l)	0.71 ± 0.13	0.79 ± 0.19	0.87 ± 0.32	0.92 ± 0.11	1.07** ± 0.09 (+51%)	0.70 ± 0.13	0.71 ± 0.14	0.94 ± 0.10	0.76 ± 0.27	0.87 ± 0.10
T_4 (nmol/l)	44.66 ±	46.88 ± 9.49	52.95 ±	40.93 ± 11.62	35.73 ± 15.07	28.45 ±	28.66 ±	39.80 ± 8.85	33.26 ± 6.79	24.63 ± 3.64

	Group mean values ± SD									
	Males (n = 5/group)					Females (n = 5/group)				
	0 ppm	100 ppm	500 ppm	2000 ppm	6000 ppm	0 ppm	100 ppm	500 ppm	2000 ppm	6000 ppm
	8.14		10.25			8.01	5.82			
TSH (µg/l)	6.57 ± 1.32	6.05 ± 1.04	7.79 ± 1.75	11.09* ± 2.80 (+69%)	10.30 ± 4.72 (+57%)	5.69 ± 0.98	6.15 ± 1.01	5.99 ± 0.69	5.67 ± 1.35	6.83 ± 2.33

From Kamp et al. (2009c)
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; SD, standard deviation; T_3, triiodothyronine; T_4, thyroxine; TSH, thyroid stimulating hormone; * $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)

Table 26. Selected haematology findings in rats administered fluxapyroxad for at least 28 days

	Group mean values ± SD									
	Males (n = 5/group)					Females (n = 5/group)				
	0	100	500	2000	6000	0	100	500	2000	6000
White blood cells (10^9/l)	4.63 ± 1.43	5.26 ± 0.75	5.16 ± 1.09	5.84 ± 0.86	4.97 ± 1.04	5.31 ± 1.22	3.30** ± 0.54 (−38%)	2.99** ± 0.79 (−44%)	3.70* ± 0.72 (−30%)	3.56 ± 0.99
Mean corpuscular haemoglobin (fmol)	1.15 ± 0.03	1.16 ± 0.03	1.17 ± 0.06	1.19 ± 0.05	1.19 ± 0.02	1.24 ± 0.04	1.18 ± 0.07	1.16* ± 0.02 (−6.4%)	1.19 ± 0.02	1.13* ± 0.05 (−8.9%)
Prothrombin time (HQT, s)	32.4 ± 3.4	32.4 ± 2.9	34.4 ± 2.5	31.3 ± 5.8	29.8 ± 5.4	31.9 ± 2.0	30.7 ± 1.1	29.1 ± 1.2	26.5** ± 1.8 (−5.4 s)	23.9** ± 1.2 (−8.0 s)

From Kamp et al. (2009c)
HQT, Hepato-Quick test; SD, standard deviation; * $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)

In a 13-week toxicity study in rats, groups of 10 Wistar rats of each sex per dose received fluxapyroxad (purity 99.6%) in the diet at a concentration of 0, 100, 500, 2000 or 6000 ppm (equal to 0, 6.1, 31.2, 126 and 407 mg/kg bw per day for males and 0, 7.3, 35.1, 144 and 424 mg/kg bw per day for females, respectively). Clinical signs were recorded at least daily, a detailed physical examination was performed weekly and functional observational battery, grip strength and motor activity assessments were performed in week 13. Ophthalmoscopic examinations were performed predosing and in animals treated at 0 and 6000 ppm on day 91. Body weights and feed consumption were recorded weekly, and haematology, plasma clinical chemistry and urine analysis were performed in week 13, prior to necropsy. All animals, including decedents, were subjected to necropsy, postmortem examination, organ weight recording and tissue preservation. Major organs and tissues from the control and high-dose groups and liver, kidneys and gross lesions from all animals were processed and examined by light microscopy. Data were analysed statistically where appropriate.

There were no treatment-related mortalities or clinical signs of toxicity. Significant decreases in body weight and body weight gain were observed in 6000 ppm males and females (Table 27). No evidence of neurotoxicity was observed in the functional observational battery. Also, no signs of neurotoxicty were noted in daily cage-side or weekly clinical examinations, and no ophthalmoscopic changes were noted.

Table 27. Mean body weight and body weight gain of rats administered fluxapyroxad for at least 91 days

	Mean values ± SD									
	Males (n = 10/group)					Females (n = 10/group)				
	0 ppm	100 ppm	500 ppm	2000 ppm	6000 ppm	0 ppm	100 ppm	500 ppm	2000 ppm	6000 ppm
Body weight (g)										
- day 0	163.6 ± 8.9	165.4 ± 5.5	161.1 ± 7.1	162.8 ± 7.0	161.7 ± 8.0	126.5 ± 5.5	128.5 ± 6.3	126.3 ± 3.2	127.2 ± 6.0	125.3 ± 5.6
- day 42	331.7 ± 20.3	350.7 ± 24.3	323.9 ± 20.3	331.6 ± 19.0	313.9 ± 27.9	204.2 ± 12.3	204.1 ± 19.5	205.3 ± 10.8	202.7 ± 13.5	188.2* ± 10.2
% change, days 0–42[a]	—	—	—	—	—	—	—	—	—	*−7.9*
- day 90	395.0 ± 24.5	419.7 ± 29.1	391.1 ± 25.7	392.8 ± 20.5	362.0* ± 35.4	227.8 ± 17.1	228.3 ± 25.0	229.8 ± 15.9	229.3 ± 13.5	198.2** ± 15.2
% change, days 0–90[a]	—	—	—	—	*−8.4*	—	—	—	—	*−13.0*
Overall body weight gain (g)	231.4 ± 22.1	254.3 ± 29.0	230.1 ± 21.5	230.0 ± 17.4	200.3* ± 34.0	101.2 ± 15.0	99.3 ± 20.0	103.5 ± 15.5	102.1 ± 10.1	72.8** ± 13.4
% change[a]	—	—	—	—	*−13.5*	—	—	—	—	*−28.1*

From Kamp et al. (2009d)

SD, standard deviation; * $P \leq 0.05$; ** $P \leq 0.01$ (Dunnett's test, two-sided)

[a] Compared with control.

In the liver, a number of changes were observed. These included changes in a number of clinical chemistry parameters (Table 28), increased absolute and relative liver weights at and above 500 ppm (Table 29) as well as histopathological changes (increased incidence and severity of centrilobular hepatocyte hypertrophy in both sexes at and above 500 ppm, which is considered adaptive and not adverse; and hepatocellular single-cell necrosis in top-dose males, which is considered clearly adverse) (Table 30). The clinical chemistry changes considered potentially indicative of adverse effects were increased serum cholesterol and triglycerides in females at and above 2000 ppm and in males at 6000 ppm and decreased prothrombin time in females at and above 500 ppm, which indicated a potential for increased blood clotting. The increase in GGT activities in males at 2000 ppm and in both sexes at 6000 ppm was regarded equivocal, as elevated GGT can be

Table 28. Selected clinical chemistry findings in rats administered fluxapyroxad for at least 91 days

	Group mean values ± SD									
	Males (*n* = 10/group)					Females (*n* = 10/group)				
	0 ppm	100 ppm	500 ppm	2000 ppm	6000 ppm	0 ppm	100 ppm	500 ppm	2000 ppm	6000 ppm
GGT (µkat/l)	0 ± 0	0 ± 0	0 ± 0	8* ± 9	262** ± 82	0 ± 0	0 ± 1	1 ± 3	1 ± 3	64** ± 39
AST (µkat/l)	2.68 ± 0.97	2.34 ± 0.81	1.87* ± 0.52 (−30%)	1.83* ± 0.48 (−32%)	1.52** ± 0.18 (−43%)	1.73 ± 0.33	1.71 ± 0.22	1.49 ± 0.21	1.28** ± 0.13 (−26%)	1.31** ± 0.25 (−24%)
ALP (µkat/l)	1.2 ± 0.20	1.15 ± 0.15	1.14 ± 0.23	1.04 ± 0.19	1.13 ± 0.16	0.54 ± 0.11	0.50 ± 0.0.06	0.46 ± 0.11	0.39** ± 0.07 (−28%)	0.46 ± 0.10
ALT (µkat/l)	1.61 ± 1.29	0.89 ± 0.13	0.88 ± 0.17	0.85 ± 0.18	0.88 ± 0.29	0.69 ± 0.12	0.60 ± 0.13	0.64 ± 0.07	0.59 ± 0.09	0.60 ± 0.07
Na^+ (mmol/l)	142.7 ± 0.8	143.4 ± 0.9	142.9 ± 1.7	142.9 ± 1.2	142.7 ± 1.1	141.8 ± 1.2	143.0* ± 0.6 (+1%)	143.2 ± 1.0* (+1%)	143.2 ± 1.5	142.2 ± 1.0
K^+ (mmol/l)	4.58 ± 0.30	4.48 ± 0.38	4.47 ± 0.18	4.33 ± 0.16	4.39 ± 0.26	4.28 ± 0.22	4.13 ± 0.19	4.12 ± 0.20	4.14 ± 0.18	4.48** ± 0.13 (+5%)
Cl^- (mmol/l)	103.5 ± 1.0	103.7 ± 1.2	103.2 ± 1.5	102.0* ± 1.4 (−1.4%)	101.5** ± 1.5 (−1.9%)	105.1 ± 1.4	106.1 ± 0.9	105 ± 0.7	103.5* ± 1.7 (−1.5%)	102.0** ± 1.2 (−2.9%)
PO_4^{3-}, inorganic (mmol/l)	1.56 ± 0.19	1.57 ± 0.18	1.60 ± 0.15	1.80** ± 0.20 (+15%)	1.79** ± 0.11 (+15%)	1.25 ± 0.22	1.23 ± 0.25	1.29 ± 0.20	1.44 ± 0.15	1.54** ± 0.17 (+23%)
Ca^{2+} (mmol/l)	2.55 ± 0.04	2.57 ± 0.03	2.60** ± 0.04 (+2%)	2.68** ± 0.06 (+5%)	2.74** ± 0.03(+7%)	2.64 ± 0.05	2.61 ± 0.08	2.68 ± 0.06	2.75** ± 0.04 (+4%)	2.85** ± 0.06 (+8%)
Urea (mmol/l)	6.62 ± 0.48	6.32 ± 0.77	6.36 ± 0.72	7.08* ± 0.25 (+7%)	7.27* ± 0.78 (+10%)	7.08 ± 0.73	6.78 ± 0.65	6.58 ± 0.62	7.36 ± 0.71	7.54 ± 0.57
Glucose (mmol/l)	5.77 ± 0.87	6.01 ± 0.63	5.60 ± 0.66	5.12* ± 0.35 (−11%)	4.67** ± 0.43 (−19%)	4.98 ± 0.53	5.22 ± 0.56	5.53 ± 0.78	5.34 ± 0.35	4.50* ± 0.36 (−9.6%)
Bilirubin, total (µmol/l)	2.40 ± 0.38	2.04 ± 0.33	1.74** ± 0.23 (−28%)	1.63** ± 0.26 (−32%)	1.81* ± 0.56 (−25%)	2.90 ± 0.43	3.01 ± 0.44	2.45* ± 0.42 (−16%)	2.45* ± 0.44 (−16%)	2.53 ± 0.39 (−13%)

Table 28 (continued)

	Group mean values ± SD									
	Males (n = 10/group)					Females (n = 10/group)				
	0 ppm	100 ppm	500 ppm	2000 ppm	6000 ppm	0 ppm	100 ppm	500 ppm	2000 ppm	6000 ppm
Protein, total (g/l)	64.74 ± 2.23	66.4 ± 1.71	66.28 ± 2.11	70.73** ± 2.63 (+9%)	72.38** ± 2.14 (+12%)	67.72 ± 4.03	68.63 ± 4.06	70.85 ± 2.96	75.69** ± 2.93 (+12%)	79.56** ± 3.76 (+17%)
Albumin (g/l)	36.45 ± 0.95	36.96 ± 0.47	37.04 ± 1.11	38.08* ± 1.24 (+4%)	39.53** ± 1.23 (+8%)	40.49 ± 2.10	40.55 ± 2.31	40.60 ± 1.81	42.09 ± 1.70	44.01** ± 1.96 (+9%)
Globulin (g/l)	28.29 ± 1.53	29.45 ± 1.54	29.24 ± 1.16	32.66** ± 1.59 (+15%)	32.85** ± 1.31 (+16%)	27.22 ± 2.24	28.08 ± 2.02	30.25** ± 1.76 (+11%)	33.60** ± 1.50 (+23%)	35.55** ± 2.52 (+31%)
Triglycerides (mmol/l)	0.62 ± 0.19	0.62 ± 0.18	0.90 ± 0.60	0.77 ± 0.24	1.15** ± 0.42 (+85%)	0.36 ± 0.06	0.41 ± 0.12	0.38 ± 0.06	0.52** ± 0.10 (+44%)	1.29** ± 0.73 (+358%)
Cholesterol (mmol/l)	1.75 ± 0.32	1.75 ± 0.27	2.00 ± 0.21	2.67** ± 0.36 (+53%)	3.90** ± 0.43 (+223%)	1.20 ± 0.24	1.64* ± 0.48 (+37%)	2.09** ± 0.45 (+74%)	2.76** ± 0.46 (+230%)	3.84** ± 0.62 (+318%)
T_3 (nmol/l)	0.73 ± 0.20	0.92* ± 0.21 (+26%)	0.96* ± 0.24 (+32%)	1.01** ± 0.19 (+38%)	1.04** ± 0.20 (+42%)	0.96 ± 0.23	1.08 ± 0.25	1.05 ± 0.13	1.33** ± 0.29 (+39%)	1.33* ± 0.38 (+39%)
T_4 (nmol/l)	42.78 ± 4.96	47.65* ± 9.60 (+11%)	50.74* ± 8.66 (+19%)	48.86 ± 5.52	42.22 ± 6.35	28.96 ± 6.25	35.31 ± 8.41	36.55* ± 8.03 (+26%)	39.59** ± 6.48 (+37%)	36.56* ± 6.83 (+26%)
TSH (µg/l)	8.12 ± 1.74	7.39 ± 1.19	9.27 ± 2.87	11.04 ± 4.45	9.23 ± 3.36	5.29 ± 0.75	5.54 ± 0.63	5.44 ± 1.73	6.47* ± 1.39 (+22%)	7.09** ± 1.87 (+34%)

From Kamp et al. (2009d)

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; SD, standard deviation; T_3, triiodothyronine; T_4, thyroxine; TSH, thyroid stimulating hormone; * $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)

Table 29. Selected mean absolute and relative organ weights of rats administered fluxapyroxad for at least 91 days

	Dietary concentration (ppm)	Mean values ± SD							
		Males (*n* = 10/group)				Females (*n* =10/group)			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
Terminal weight (g)	0	368.8 ± 24.2	—	—	—	212.5 ± 14.9	—	—	—
	100	392.5* ± 26.4	(+6.4)	—	—	210.4 ± 23.7	(−1.0)	—	—
	500	361.2 ± 23.9	(−2.1)	—	—	213.3 ± 14.1	(+0.4)	—	—
	2000	363.9 ± 20.4	(−1.3)	—	—	211.0 ± 13.6	(−0.7)	—	—
	6000	331.9* ± 35.8	(−10.0)	—	—	185.0** ± 14.2	(−12.9)	—	—
Adrenal glands (mg)	0	63.0 ± 7.5	—	0.017 ± 0.002	—	70.5 ± 7.8	—	0.033 ± 0.003	—
	100	64.5 ± 4.4	(+2.4)	0.016 ± 0.001	(−3.9)	79.4 ± 14.5	(+12.7)	0.038** ± 0.003	(+13.1)
	500	59.4 ± 7.2	(−5.7)	0.016 ± 0.002	(−4.0)	74.5 ± 10.2	(+5.7)	0.035 ± 0.005	(+5.3)
	2000	66.3 ± 11.2	(+5.2)	0.018 ± 0.003	(+6.1)	74.3 ± 9.92	(+5.4)	0.035 ± 0.003	(+5.8)
	6000	67.0 ± 9.2	(+6.3)	0.02** ± 0.002	(+18.0)	58.8** ± 8.08	(−16.6)	0.032 ± 0.002	(−4.5)
Brain (mg)	0	2.09 ± 0.06	—	0.568 ± 0.030	—	1.95 ± 0.09	—	0.92 ± 0.045	—
	100	2.18 ± 0.12	(+4.5)	0.557 ± 0.036	(−1.9)	1.93 ± 0.13	(−1.1)	0.922 ± 0.057	(+0.2)
	500	2.08 ± 0.09	(−0.4)	0.578 ± 0.036	(+1.8)	1.98 ± 0.07	(+1.4)	0.931 ± 0.069	(+1.2)
	2000	2.11 ± 0.06	(+1.1)	0.581 ± 0.023	(+2.3)	1.97 ± 0.08	(+1.1)	0.938 ± 0.062	(+2.0)
	6000	2.08 ± 0.08	(−0.5)	0.631** ± 0.053	(+11.1)	1.87 ± 0.07	(−4.2)	1.01** ± 0.07	(+10.2)

Table 29 (continued)

	Dietary concentration (ppm)	Mean values ± SD							
		Males (*n* = 10/group)				Females (*n* =10/group)			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
Heart (mg)	0	1.05 ± 0.08	—	0.287 ± 0.026	—	0.687 ± 0.072	—	0.323 ± 0.026	—
	100	1.17 ± 0.18	(+10.6)	0.296 ± 0.030	(+3.1)	0.697 ± 0.080	(+1.5)	0.332 ± 0.035	(+2.8)
	500	1.09 ± 0.14	(+3.6)	0.302 ± 0.036	(+5.2)	0.691 ± 0.054	(+0.6)	0.324 ± 0.019	(+0.3)
	2000	1.07 ± 0.08	(+1.7)	0.295 ± 0.017	(+2.8)	0.688 ± 0.043	(+0.1)	0.326 ± 0.015	(+0.9)
	6000	1.11 ± 0.14	(+5.6)	0.337** ± 0.039	(+17.4)	0.629 ± 0.060	(−8.4)	0.34 ± 0.017	(+5.3)
Kidneys (mg)	0	2.36 ± 0.26	—	0.642 ± 0.083	—	1.41 ± 0.12	—	0.664 ± 0.036	—
	100	2.55 ± 0.30	(+8.2)	0.652 ± 0.083	(+1.6)	1.51 ± 0.23	(+7.4)	0.719 ± 0.064	(+8.3)
	500	2.44 ± 0.26	(+3.5)	0.677 ± 0.073	(+5.5)	1.48 ± 0.10	(+5.1)	0.697 ± 0.059	(+5.0)
	2000	2.48 ± 0.22	(+5.2)	0.681 ± 0.043	(+6.1)	1.55* ± 0.17	(+10.1)	0.735 ± 0.06	(+10.7)
	6000	2.28 ± 0.28	(−3.2)	0.691 ± 0.087	(+7.6)	1.31 ± 0.11	(−7.0)	0.709 ± 0.044	(+6.8)
Liver (mg)	0	8.11 ± 0.54	—	2.202 ± 0.148	—	4.95 ± 0.38	—	2.33 ± 0.17	—
	100	8.92* ± 0.75	(+10.0)	2.271 ± 0.110	(+3.1)	5.21 ± 0.71	(+5.4)	2.48 ± 0.22	(+6.3)
	500	9.34** ± 0.71	(+15.3)	2.589** ± 0.135	(+17.6)	5.60** ± 0.45	(+13.3)	2.63** ± 0.20	(+12.8)
	2000	12.1** ± 1.5	(+48.9)	3.308** ± 0.272	(+50.2)	6.96** ± 0.59	(+40.8)	3.30** ± 0.13	(+41.4)

	Dietary concentration (ppm)	Mean values ± SD							
		Males (n = 10/group)				Females (n =10/group)			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
	6000	14.4** ± 1.3	(+77.8)	4.371** ± 0.461	(+98.5)	7.77** ± 0.68	(+57.1)	4.20** ± 0.23	(+80.1)
Testes (mg)	0	3.39 ± 0.25	—	0.896 ± 0.090	—	—	—	—	—
	100	3.53 ± 0.37	(+7.2)	0.901 ± 0.105	(+0.6)	—	—	—	—
	500	3.37 ± 0.31	(+2.5)	0.935 ± 0.082	(+4.4)	—	—	—	—
	2000	3.47 ± 0.35	(+5.4)	0.953 ± 0.071	(+6.4)	—	—	—	—
	6000	3.54 ± 0.44	(+7.7)	1.075** ± 0.153	(+20.0)	—	—	—	—
Thymus (mg)	0	269.0 ± 43.0	—	0.073 ± 0.010	—	275.2 ± 33.7	—	0.13 ± 0.01	—
	100	270.0 ± 26.6	(+0.4)	0.069 ± 0.008	(−5.3)	277.4 ± 45.3	(+0.8)	0.132 ± 0.016	(+1.5)
	500	301.9 ± 53.1	(+12.2)	0.083* ± 0.012	(+14.3)	278.9 ± 52.7	(+1.3)	0.13 ± 0.02	(+0.2)
	2000	278.5 ± 30.2	(+3.5)	0.077 ± 0.007	(+5.0)	269.5 ± 29.8	(−2.1)	0.128 ± 0.016	(−1.4)

From Kamp et al. (2009d)

SD, standard deviation; * $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)

[a] Compared with control. Values may not calculate exactly due to rounding.

[b] Percentage of body weight.

Table 30. Incidence of selected macropathological and histopathological lesions in rats administered fluxapyroxad for at least 91 days

	Males (n = 10/group)					Females (n = 10/group)				
	0 ppm	100 ppm	500 ppm	2000 ppm	6000 ppm	0 ppm	100 ppm	500 ppm	2000 ppm	6000 ppm
Macropathology										
Liver										
No. examined	10	10	10	10	10	10	10	10	10	10
Discoloration	0	0	0	0	0	0	0	0	0	10
Histopathology										
Kidney										
No. examined	10	—	—	1	10	10	9	10	10	10
Pigment storage, tubular	0	—	—	0	0	3	1	0	3	10
						[1.0]	[1.0]		[1.0]	[1.7]
Liver										
No. examined	10	10	10	10	10	10	9	10	10	10
Hypertrophy, central (zone 3)	0	2	9	10	10	0	0	9	10	10
		[1.0]	[1.0]	[2.4]	[2.2]			[1.4]	[2.5]	[3.0]
Necrosis, central (zone 3), single cell	0	0	0	0	9	0	0	0	0	—
					[1.8]					
Thyroid										
No. examined	10	10	10	10	10	10	9	10	10	10
Hypertrophy/hyperplasia, follicular	2	1	2	8	8	0	1	4	5	6
	[1.0]	[1.0]	[1.0]	[1.0]	[1.0]		[1.0]	[1.0]	[1.0]	[1.0]

From Kamp et al. (2009d)

[a] [ ] mean severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked (Grade 4) and massive/severe (Grade 5). The mean severity is the sum of the gradings divided by the incidence.

associated with both adaptive and adverse effects. Changes in thyroid hormone levels were observed in males at and above 100 ppm (Table 28), with thyroid follicular cell hypertrophy/hyperplasia present at and above 2000 ppm (Table 30). In females, both changes in thyroid hormone levels and thyroid follicular hypertrophy/hyperplasia were observed at and above 500 ppm. Additionally, an increased tubular deposition of pigment, indicative of a mild tubulonephrosis, was observed in kidneys of top-dose females and was not considered adverse.

The NOAEL was 500 ppm (equal to 31.2 mg/kg bw per day) in males and 100 ppm (equal to 7.3 mg/kg bw per day) in females, based on thyroid follicular hypertrophy and hyperplasia seen at 2000 ppm (equal to 126 mg/kg bw per day) in males and 500 ppm (equal to 35.1 mg/kg bw per day) in females (Kamp et al., 2009d).

Dogs

In a short-term oral toxicity study, five Beagle dogs of each sex per dose were administered fluxapyroxad (purity 99.7%) in the diet for 28 days at a concentration of 0, 2500, 7500 or 20 000 ppm (equal to doses of 0, 74, 211 and 521 mg/kg bw per day for males and 0, 85, 230 and 503 mg/kg bw per day for females, respectively). The animals were subjected weekly to a detailed clinical examination and body weight and feed consumption recording. Blood samples for haematology and plasma clinical chemistry were withdrawn and urine samples were collected from all animals predosing and in week 4. The animals were sacrificed after 28 days of treatment and subjected to necropsy and postmortem examination of major organs and tissues. Organs were weighed, and a full range of tissues was preserved, processed and examined by light microscopy.

There were no deaths, and no effects on urine analysis or gross lesions at necropsy were observed at any dose level. There were no treatment-related effects in the semiquantitative observations made in the home cage and in the open field.

Impaired body weight development and decreased feed consumption were observed at 7500 ppm and above (Tables 31 and 32). Vomiting was observed in five males and four females at 7500 and 20 000 ppm and is considered adverse. There were no treatment-related changes in haematological parameters. Changes in a number of clinical chemistry parameters (Table 33) as well as a slight, but statistically significant, increase in relative liver weights at and above 7500 ppm were observed. The clinical chemistry changes consisted of increased ALP, serum GGT (considered potentially adverse) and triglyceride levels and decreased total protein, albumin, cholesterol, total bilirubin and calcium levels. These findings were partially observed down to the low dose level. In addition, single mid- and high-dose males displayed increased ALT and aspartate aminotransferase (AST) activities. Although these changes did not result in a significant change of mean or median values, they were considered to be treatment related. Absolute and relative thymus weights were decreased in all treated groups (Table 34); however, the values were within the range of the historical control data provided (Table 35). There were no treatment-related macropathological or histopathological findings in the liver or other organs and tissues at any dose level.

The NOAEL was 2500 ppm (equal to 74 mg/kg bw per day for males and 85 mg/kg bw per day for females), based on vomiting and clinical chemistry changes seen at 7500 ppm (equal to 211 mg/kg bw per day for males and 230 mg/kg bw per day for females) (Hempel et al., 2009a).

In a short-term oral toxicity study, five Beagle dogs of each sex per dose were administered fluxapyroxad (purity 99.7%) in the diet for 13 weeks at a concentration of 0, 300, 1500 or 10 000 (males)/7500 (females) ppm (equal to dose levels of 0, 9.0, 45 and 295 mg/kg bw per day for males and 0, 10, 51 and 238 mg/kg bw per day for females, respectively). The animals were subjected weekly to a detailed clinical examination and body weight and feed consumption recording. Ophthalmological examinations were performed on all animals predosing and in week 13. Blood samples for haematology and plasma clinical chemistry were withdrawn and urine samples were collected from all animals predosing and in weeks 7 and 13. The animals were sacrificed after 91 days of treatment and subjected to necropsy and postmortem examination of major organs and tissues.

Table 31. Body weight and body weight gain for dogs administered fluxapyroxad for 28 days

	Mean values ± SD							
	Males (n = 5/group)				Females (n = 5/group)			
	0 ppm	2500 ppm	7500 ppm	20 000 ppm	0 ppm	2500 ppm	7500 ppm	20 000 ppm
Body weight (kg)								
- day 0	13.1 ± 1.9	13.2 ± 2.1	13.3 ± 1.2	13.3 ± 1.5	11.3 ± 1.6	11.2 ± 1.2	11.1 ± 1.2	11.0 ± 1.6
- day 28	13.8 ± 1.7	13.9 ± 1.9	13.8 ± 1.5	12.9 ± 1.7	11.9 ± 1.4	12.0 ± 1.1	11.3 ± 1.4	10.6 ± 1.8
% change[a,b]	—	*+0.4*	*−0.1*	*−6.5*	—	*+1.0*	*−4.4*	*−10.5*
Overall body weight gain (g)	0.7 ± 0.3	0.7 ± 0.1	0.5 ± 0.6	−0.3** ± 0.3	0.6 ± 0.3	0.8 ± 0.2	0.3 ± 0.5	−0.4** ± 0.4
% change[a,b]	—	—	*−29*	*−142*	—	*+33*	*−50*	*−167*

From Hempel et al. (2009a)
** $P \leq 0.01$ (Dunnett's test, two-sided)
[a] Compared with control.
[b] Calculated from the weekly means.

Table 32. Feed efficiency in dogs administered fluxapyroxad for 28 days

Dietary concentration (ppm)	Mean feed efficiency ± SD							
	Males (n = 5/group)				Females (n = 5/group)			
	Day 7	Day 14	Day 21	Day 28	Day 7	Day 14	Day 21	Day 28
0 (control)	4.6 ± 2.0	3.9 ± 2.9	1.4 ± 2.0	2.5 ± 3.2	1.7 ± 3.0	6.8 ± 2.3	0.7 ± 3.0	1.0 ± 2.1
2500	4.6 ± 2.4	3.6 ± 2.8	0.7 ± 1.6	3.9 ± 1.5	4.0 ± 1.8	3.9 ± 2.9	3.6 ± 1.8	2.1 ± 1.5
7500	0.2 ± 2.1	4.2 ± 4.2	0.8 ± 5.5	4.1 ± 3.1	−2.0 ± 3.6	4.9 ± 4.7	0.7 ± 3.7	−0.5 ± 6.6
20 000	−16.1 ± 13.6	6.9 ± 4.7	−3.5 ± 4.5	0.9 ± 5.3	−19.8 ± 8.4	5.3 ± 4.3	−6.2 ± 7.0	6.1 ± 2.2

From Hempel et al. (2009a)

Table 33. Selected clinical chemistry findings of dogs administered fluxapyroxad for 27 days (males) or 28 days (females)

	Group mean values ± SD							
	Males (n = 5/group)				Females (n = 5/group)			
	0 ppm	2500 ppm	7500 ppm	20 000 ppm	0 ppm	2500 ppm	7500 ppm	20 000 ppm
ALT (µkat/l)	0.63 ± 0.18	0.56 ± 0.22	1.31 ± 1.82	2.62 ± 4.22	0.59 ± 0.18	0.62 ± 0.28	0.56 ± 0.10	0.66 ± 0.60
AST (µkat/l)	0.53 ± 0.11	0.57 ± 0.18	0.69 ± 0.37	1.23 ± 1.36	0.46 ± 0.08	0.68 ± 0.14	0.54 ± 0.08	0.59 ± 0.24
ALP (µkat/l)	1.75 ± 0.44	3.06* ± 0.69 (+75%)	7.81** ± 6.59 (+340%)	11.37** ± 6.23 (+550%)	1.88 ± 0.79	3.41* ± 0.76 (+81%)	6.94** ± 2.62 (+270%)	12.29** ± 7.03 (+540%)

	Group mean values ± SD							
	Males (*n* = 5/group)				Females (*n* = 5/group)			
	0 ppm	2500 ppm	7500 ppm	20 000 ppm	0 ppm	2500 ppm	7500 ppm	20 000 ppm
GGT (μkat/l)	38 ± 10	38 ± 9	55 ± 12	81** ± 18 (+113%)	37 ± 5	47* ± 6 (+27%)	55** ± 6 (+49%)	73** ± 21 (+97%)
Protein, total (g/l)	57.33 ± 1.98	53.83 ± 2.27	52.44* ± 3.18 (−9%)	52.13** ± 1.05 (−10%)	57.5 ± 4.00	52.02 ± 0.99	50.33* ± 2.19 (−12%)	50.19* ± 3.44 (−13%)
Albumin (g/l)	33.93 ± 0.31	31.84** ± 1.11 (−6.2%)	29.40** ± 2.96 (−13%)	29.36** ± 1.73 (−13%)	35.21 ± 2.15	31.96** ± 0.94 (−9.2%)	29.72** ± 1.87 (−16%)	29.79** ± 2.09 (−15%)
Triglycerides (mmol/l)	0.30 ± 0.06	0.27 ± 0.03	0.40 ± 0.04	0.50* ± 0.13 (+67%)	0.38 ± 0.08	0.29 ± 0.06	0.30 ± 0.05	0.54* ± 0.12 (+42%)
Cholesterol (mmol/l)	3.79 ± 0.55	2.86 ± 0.77	2.50 ± 0.94	2.52 ± 0.84	3.92 ± 0.81	2.86 ± 0.27	2.29* ± 0.55 (−42%)	2.58* ± 0.55 (−34%)
Bilirubin, total (μmol/l)	2.67 ± 0.61	2.84 ± 0.63	2.27 ± 0.39	2.94 ± 0.44	4.27 ± 0.80	2.37** ± 0.35 (−44%)	2.40** ± 0.83 (−44%)	2.72** ± 0.64 (−36%)
Ca^{2+} (mmol/l)	2.78 ± 0.08	2.75 ± 0.06	2.70 ± 0.08	2.66 ± 0.05	2.77 ± 0.06	2.71 ± 0.05	2.66* ± 0.08 (−4.0%)	2.63* ± 0.08 (−5.0%)

From Hempel et al. (2009a)

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; SD, standard deviation; * $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)

Table 34. Selected mean absolute and relative organ weights of dogs administered fluxapyroxad for 28 days

	Dietary concentration (ppm)	Mean weights ± SD							
		Males (*n* = 5/group)				Females (*n* = 5/group)			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
Terminal weight (kg)	0	14.06 ± 1.72	—	—	—	12.08 ± 1.33	—	—	—
	2500	14.1 ± 2.0	(+0.3)	—	—	12.12 ± 1.13	(+0.3)	—	—
	7500	14.0 ± 1.6	(−0.4)	—	—	11.64 ± 1.41	(−3.6)	—	—
	20 000	12.88 ± 1.73	(−8.4)	—	—	10.86 ± 1.79	(−10.1)	—	—
Kidneys (g)	0	63.7 ± 13.2	—	0.45 ± 0.07	—	51.17 ± 3.14	—	0.426 ± 0.028	—
	2500	60.07 ± 8.18	(−5.6)	0.429 ± 0.05	(−4.9)	49.19 ± 4.73	(−3.9)	0.406 ± 0.023	(−4.7)
	7500	70.43 ± 11.27	(+10.6)	0.504 ± 0.062	(+11.8)	52.98 ± 4.84	(+3.5)	0.461 ± 0.077	(+8.2)

Table 34 (continued)

	Dietary concentration (ppm)	Mean weights ± SD							
		Males (n = 5/group)				Females (n = 5/group)			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
	20 000	58.61 ± 7.69	(−7.9)	0.458 ± 0.062	(+1.6)	52.9 ± 8.3	(+3.4)	0.489* ± 0.037	(+14.8)
Liver (g)	0	371.5 ± 62.0	—	2.64 ± 0.24	—	345.8 ± 36.4	—	2.868 ± 0.183	—
	2500	389.7 ± 51.6	(+4.9)	2.78 ± 0.244	(+5.3)	332.8± 15.59	(−3.8)	2.758 ± 0.19	(−3.8)
	7500	468.2 ± 80.5	(+26.0)	3.34* ± 0.37	(+26.6)	391.7 ± 59.7	(+13.2)	3.381 ± 0.514	(+17.9)
	20 000	437.1 ± 78.9	(+17.7)	3.38** ± 0.235	(+28.2)	380.4 ± 74.7	(+10.0)	3.49** ± 0.26	(+21.8)
Thymus (g)	0	16.1 ± 3.8	—	0.117 ± 0.034	—	17.7 ± 5.6	—	0.148 ± 0.053	—
	2500	12.9 ± 4.9	(−19.6)	0.09 ± 0.03	(−23.1)	11.75* ± 1.18	(−33.7)	0.098* ± 0.013	(−33.8)
	7500	10.9 ± 5.2	(−32.3)	0.076 ± 0.032	(−35.0)	8.46* ± 3.79	(−52.3)	0.071** ± 0.025	(−52.0)
	20 000	6.29** ± 2.16	(−60.9)	0.049** ± 0.015	(−58.1)	7.14* ± 3.4	(−59.7)	0.065** ± 0.024	(−56.1)
Uterus (g)	0	—	—	—	—	7.83 ± 7.5	—	0.064 ± 0.059	—
	2500	—	—	—	—	5.62 ± 2.06	(−28.3)	0.047 ± 0.018	(−26.6)
	7500	—	—	—	—	3.11 ± 2.4	(−60.3)	0.025 ± 0.016	(−60.9)
	20 000	—	—	—	—	1.84** ± 0.95	(−76.5)	0.016** ± 0.007	(−75.0)

From Hempel et al. (2009a)
SD, standard deviation; * $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)
[a] Compared with control. Values may not calculate exactly due to rounding.
[b] Percentage of body weight.

Table 35. Historical control data for absolute and relative thymus weights

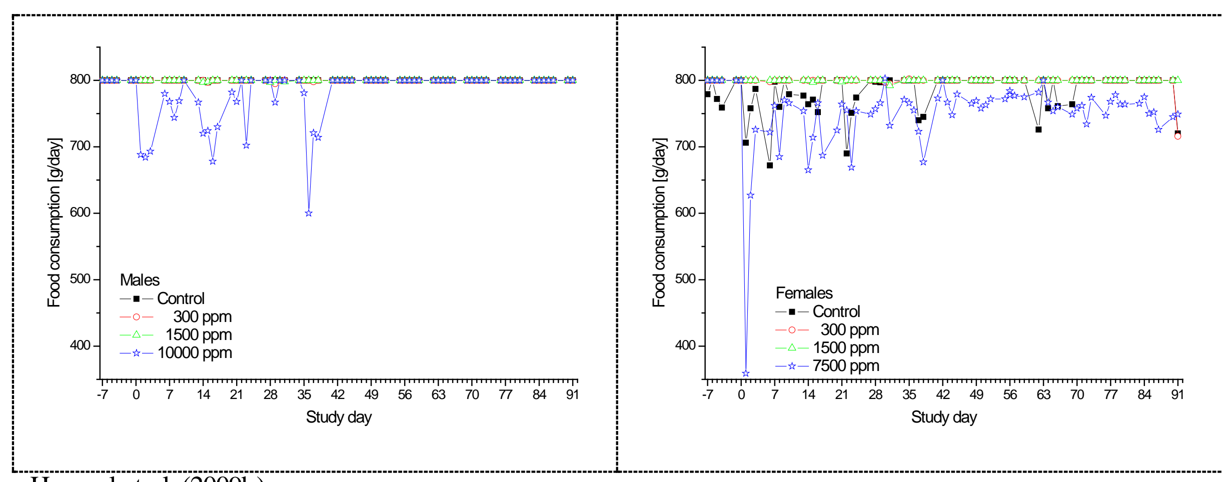
Study No.	Application	Study start	Study end	Mean thymus weight ± SD			
				Males absolute	Males relative	Females absolute	Females relative
01144	Capsule	03/2004	04/2004	9.280 ± 5.012	0.066 ± 0.034	11.920 ± 2.312	0.089 ± 0.021
01164	Capsule	02/2005	03/2005	8.210 ± 2.997	0.059 ± 0.022	7.098 ± 1.511	0.057 ± 0.013
04064	Feeding	09/2005	10/2005	6.600 ± 1.684	0.043 ± 0.015	8.773 ± 3.107	0.060 ± 0.020
05077 (fluxapyroxad)	Feeding	04/2007	05/2007	16.09 ± 3.80	0.117 ± 0.034	17.738 ± 5.578	0.148 ± 0.053

From Hempel et al. (2009a)
SD, standard deviation

Organs were weighed, and a full range of tissues was preserved, processed and examined by light microscopy.

Decreased feed consumption was observed in two high-dose males and two high-dose females (Figure 3). This was accompanied by decreased body weight development in one high-dose female only (Table 36). Furthermore, vomiting was recorded in all male and female dogs of the high-dose group during the first 2 days of treatment. There were no deaths, and no effects on ocular architecture, haematology (Table 37), urine analysis or gross lesions at necropsy were observed at any dose level. There were no treatment-related effects in the semiquantitative observations made in the home cage or in the open field.

Figure 3. Mean daily feed consumption in dogs administered fluxapyroxad for at least 91 days



From Hempel et al. (2009b)

Table 36. Mean body weight and body weight gain of dogs administered fluxapyroxad for 90 days

	Mean weights ± SD							
	Males (n = 5/group)				Females (n = 5/group)			
	0 ppm	300 ppm	1500 ppm	10 000 ppm	0 ppm	300 ppm	1500 ppm	7500 ppm
Body weight (kg)								
- day 0	11.60 ± 1.4	11.9 ± 1.3	11.7 ± 1.6	11.8 ± 1.0	10.5 ± 1.2	10.2 ± 1.1	10.3 ± 1.1	10.5 ± 1.3
- day 91	13.9 ± 1.4	14.0 ± 0.8	14.6 ± 1.1	14.20 ± 0.7	13.1 ± 1.5	12.5 ± 1.1	12.6 ± 1.2	12.7 ± 1.6
% change[a]	—	*+0.6*	*+4.4*	*+1.9*	—	*−4.4*	*−3.8*	*−3.1*
Overall body weight gain (g)								
- days 0–91	2.3 ± 0.6	2.1 ± 0.7	2.8 ± 0.7	2.4 ± 0.5	2.5 ± 0.7	2.2 ± 0.5	2.3 ±.2	2.2 ± 0.5
% change[a]	—	*−8.7*	*+22*	*+4.3*	—	*−8.0*	*−8.0*	*−8.0*

From Hempel et al. (2009b)
SD, standard deviation
[a] Compared with control.

Table 37. Selected haematology findings of dogs administered fluxapyroxad for 90 days

	Week	Group mean values ± SD							
		Males (*n* = 5/group)				Females (*n* = 5/group)			
		0 ppm	300 ppm	1500 ppm	10 000 ppm	0 ppm	300 ppm	1500 ppm	7500 ppm
Mean corpuscular haemoglobin (fmol)	6	1.39 ± 0.07	1.41 ± 0.04	1.41 ± 0.06	1.45 ± 0.01	1.43 ± 0.02	1.42 ± 0.05	1.41 ± 0.02	1.42 ± 0.03
	13	1.39 ± 0.06	1.41 ± 0.04	1.42 ± 0.06	1.48* ± 0.02	1.42 ± 0.03	1.43 ± 0.05	1.42 ± 0.02	1.47 ± 0.03
Haematocrit (1/1)	6	0.400 ± 0.023	0.403 ± 0.18	0.395 ± 0.025	0.413 ± 0.032	0.449 ± 0.027	0.407* ± 0.024	0.411 ± 0.017	0.427 ± 0.007
	13	0.428 ± 0.040	0.434 ± 0.017	0.431 ± 0.026	0.429 ± 0.030	0.452 ± 0.023	0.429 ± 0.020	0.434 ± 0.040	0.430 ± 0.019
Platelets (10^9/l)	6	359 ± 62	345 ± 73	322 ± 73	438 ± 72	350 ± 57	349 ± 91	368 ± 74	400 ± 54
	13	373 ± 81	327 ± 62	347 ± 80	432 ± 42	315 ± 30	308 ± 96	344 ± 72	445** ± 63
White blood cells (10^9/l)	6	11.32 ± 1.12	13.81 ± 3.43	11.30 ± 1.68	10.08 ± 2.01	11.76 ± 2.69	11.58 ± 1.70	10.56 ± 0.95	11.72 ± 2.20
	13	11.92	11.51	10.53	9.46	10.94	11.26	10.49	11.57
Large unstained cells (10^9/l)	6	0.06 ± 0.01	0.06 ± 0.02	0.05 ± 0.02	0.04* ± 0.00	0.05 ± 0.01	0.06 ± 0.01	0.05 ± 0.02	0.04 ± 0.02
	13	0.02 ± 0.01	0.04 ± 0.01	0.03 ± 0.02	0.02 ± 0.01	0.04 ± 0.02	0.03 ± 0.02	0.04 ± 0.01	0.02 ± 0.00
Neutrophils (%)	6	52.6 ± 5.0	58.1 ± 9.4	54.2 ± 5.8	46.2 ± 6.1	52.0 ± 4.6	50.8 ± 4.9	49.2 ± 2.4	49.4 ± 5.2
	13	60.8 ± 5.6	62.4 ± 6.4	60.1 ± 4.1	48.9* ± 4.0	54.2 ± 4.4	57.5 ± 5.6	54.4 ± 2.5	52.9 ± 4.6
Lymphocytes (%)	6	38.5 ± 3.7	33.8 ± 9.3	37.5 ± 5.8	45.1 ± 5.9	40.5 ± 4.8	42.4 ± 4.3	44.0 ± 2.4	43.5 ± 5.8
	13	30.9	30.0	31.9	40.4**	38.1	36.0	38.1	39.6
Basophils (%)	6	0.7 ± 0.2	0.8 ± 0.3	0.8 ± 0.3	0.8 ± 0.3	0.9 ± 0.5	0.8 ± 0.1	0.9 ± 0.2	0.8 ± 0.2
	13	0.9 ± 0.3	1.2 ± 0.3	1.3 ± 0.2	1.6* ± 0.2	1.0 ± 0.4	0.9 ± 0.2	1.1 ± 0.3	0.9 ± 0.3

From Hempel et al. (2009b)

SD, standard deviation; * $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)

The liver was identified as the major target organ, as indicated by changes in a number of clinical chemistry parameters at 1500 and/or 10 000/7500 ppm (Table 38) as well as statistically significant changes in absolute and/or relative liver weights at 10 000 ppm in males and 7500 ppm in females (Table 39). The clinical chemistry changes considered potentially adverse were increased ALP and GGT activities in both sexes at the high dose. Increased absolute and/or relative liver weights in both sexes were considered adaptive and not adverse, as there were no histopathological correlates.

Table 38. Selected clinical chemistry findings in dogs administered fluxapyroxad for 90 days

	Week	Group mean values ± SD							
		Males (*n* = 5/group)				Females (*n* = 5/group)			
		0 ppm	300 ppm	1500 ppm	10 000 ppm	0 ppm	300 ppm	1500 ppm	7500 ppm
AST (µkat/l)	6	0.54 ± 0.06	0.74 ± 0.20	0.55 ± 0.09	0.52 ± 0.07	0.52 ± 0.07	0.55 ± 0.13	0.53 ± 0.09	0.52 ± 0.05
	13	0.51 ± 0.06	0.77* ± 0.22	0.51 ± 0.09	0.57 ± 0.12	0.49 ± 0.14	0.48 ± 0.11	0.47 ± 0.08	0.52 ± 0.11
ALT (µkat/l)	6	0.58 ± 0.02	0.79 ± 0.32	0.55 ± 0.20	0.47 ± 0.15	0.56 ± 0.15	0.62 ± 0.15	0.43 ± 0.11	0.63 ± 0.22
	13	0.75 ± 0.24	0.90 ± 0.20	0.64 ± 0.11	0.65 ± 0.16	0.63 ± 0.12	0.79 ± 0.17	0.55 ± 0.16	1.23 ± 0.75
ALP (µkat/l)	6	2.26 ± 0.56	1.91 ± 0.78	1.79 ± 0.40	5.83 ± 2.18 (+160%)	1.53 ± 0.42	1.45 ± 0.36	1.88 ± 0.28	5.67** ± 2.92 (+270%)
	13	2.01 ± 0.85	1.65 ± 0.67	1.65 ± 0.47	6.85* ± 2.90 (+240%)	1.44 ± 0.43	1.36 ± 0.41	1.82 ± 0.34	8.03* ± 5.24 (+450%)
GGT (µkat/l)	6	45 ± 11	52 ± 14	59 ± 10	65 ± 8	54 ± 4	37 ± 14	49 ± 10	66* ± 9 (+22%)
	13	47 ± 8	62 ± 12	53 ± 8	85** ± 7 (+80%)	50 ± 7	41 ± 13	47 ± 3	90* ± 27 (+80%)
Protein, total (g/l)	6	59.03 ± 3.30	57.23 ± 2.66	55.48 ± 2.04	51.65* ± 2.90 (−13%)	56.30 ± 0.64	54.49 ± 2.38	53.24** ± 1.67 (−5.4%)	49.68** ± 2.98 (−12%)
	13	63.07 ± 3.13	59.59 ± 2.91	58.30* ± 2.11 (−7.6%)	54.00** ± 2.41 (−14%)	59.44 ± 1.42	57.88 ± 3.08	54.86** ± 1.87 (−7.7%)	51.67* ± 5.34 (−13%)
Albumin (g/l)	6	34.46 ± 1.84	33.06 ± 2.03	31.65* ± 0.97 (−8%)	28.15** ± 1.90 (−18%)	34.76 ± 0.97	33.03* ± 0.80 (−5.0%)	32.38* ± 0.96 (−6.8%)	29.01** ± 1.89 (−17%)
	13	35.75 ± 1.79	34.58 ± 1.95	33.23* ± 0.90 (−7%)	29.12** ± 2.35 (−19%)	35.82 ± 1.04	34.87 ± 1.16	32.90** ± 0.93 (−8.2%)	28.66** ± 4.41 (−20%)
Triglycerides (mmol/l)	6	0.33 ± 0.05	0.28 ± 0.08	0.34 ± 0.08	0.43* ± 0.05 (+30%)	0.38 ± 0.09	0.31 ± 0.03	0.26 ± 0.07	0.48 ± 0.10
	13	0.35 ± 0.10	0.22 ± 0.05	0.28 ± 0.06	0.39 ± 0.03	0.46 ± 0.16	0.30* ± 0.05 (−35%)	0.23** ± 0.06 (−50%)	0.41 ± 0.13
Cholesterol (mmol/l)	6	3.72 ± 0.31	3.38 ± 0.51	3.25* ± 0.25 (−13%)	2.82** ± 0.37 (−24%)	3.54 ± 0.41	3.24 ± 0.22	2.88 ± 0.45	2.44 ± 0.87
	13	3.82 ± 0.20	3.37 ± 0.48	3.32** ± 0.16 (−13%)	3.02** ± 0.34 (−21%)	4.51 ± 1.07	3.59 ± 0.52	3.05* ± 0.44 (−33%)	2.82* ± 0.95 (−37%)
Urea (mmol/l)	6	5.62 ± 1.17	4.54 ± 0.60	3.97* ± 0.35 (−29%)	3.61* ± 0.60 (−36%)	5.03 ± 1.26	4.72 ± 0.42	4.83 ± 0.81	4.34 ± 0.80
	13	5.57 ± 0.99	4.85 ± 0.83	4.09 ± 0.49	4.08 ± 0.78	5.63 ± 0.84	4.70 ± 0.56	4.67 ± 0.63	4.37 ± 1.02

Table 38 (continued)

	Week	Group mean values ± SD							
		Males (n = 5/group)				Females (n = 5/group)			
		0 ppm	300 ppm	1500 ppm	10 000 ppm	0 ppm	300 ppm	1500 ppm	7500 ppm
Bilirubin, total (µmol/l)	6	3.22 ± 0.09	2.82 ± 0.71	2.79 ± 0.22	2.17 ± 0.72	3.44 ± 0.74	3.06 ± 0.78	2.77 ± 0.49	1.74** ± 0.32 (−49%)
	13	2.91 ± 0.25	3.14 ± 0.74	2.85 ± 0.30	2.29** ± 0.37 (−21%)	3.77 ± 0.40	3.51\ ± 0.51	3.44 ± 0.50	2.49** ± 0.41 (−34%)
Ca^{2+} (mmol/l)	6	2.78 ± 0.05	2.70 ± 0.09	2.70 ± 0.04	2.64* ± 0.06 (−5%)	2.75 ± 0.08	2.67 ± 0.04	2.66 ± 0.09	2.60** ± 0.03 (−5.5%)
	13	2.77 ± 0.09	2.70 ± 0.08	2.70 ± 0.03	2.62* ± 0.06 (−5.4%)	2.77 ± 0.06	2.74 ± 0.06	2.71 ± 0.07	2.62** ± 0.08 (−5.4%)
PO_4^{3-} (mmol/l)	6	1.83 ± 0.10	1.68 ± 0.11	1.73 ± 0.11	1.87 ± 0.06	1.92 ± 0.32	1.77 ± 0.12	1.72 ± 0.15	1.85 ± 0.18
	13	1.54 ± 0.16	1.52 ± 0.23	1.50 ± 0.09	1.79** ± 0.07 (+16%)	1.69 ± 0.22	1.55 ± 0.13	1.54 ± 0.12	1.75 ± 0.18

From Hempel et al. (2009b)
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; SD, standard deviation; * $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)

Table 39. Selected mean absolute and relative organ weights of dogs administered fluxapyroxad for 90 days

	Dietary concentration (ppm)	Mean weight ± SD							
		Males (n = 5/group)				Females (n = 5/group)			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
Terminal weight (kg)	0	13.88 ± 1.4	—	—	—	13.14 ± 1.52	—	—	—
	300	13.92 ± 0.84	(+0.3)	—	—	12.38 ± 0.94	(−5.8)	—	—
	1500	14.54 ± 1.18	(+4.8)	—	—	12.62 ± 1.11	(−4.0)	—	—
	10 000/7500	14.2 ± 0.7	(+2.3)	—	—	12.76 ± 1.48	(−2.9)	—	—
Kidneys (g)	0	59.90 ± 5.07	—	0.436 ± 0.066	—	53.14 ± 6.25	—	0.406 ± 0.032	—
	300	69.01* ± 4.47	(+15.2)	0.497 ± 0.035	(+14.0)	54.28 ± 2.41	(+2.1)	0.441 ± 0.045	(+8.6)
	1500	72.00 ± 8.57	(+20.2)	0.498 ± 0.079	(+14.2)	58.15 ± 9.09	(+9.4)	0.461 ± 0.065	(+13.5)
	10 000/7500	74.14* ± 11.63	(+23.8)	0.522 ± 0.077	(+19.7)	59.89 ± 9.69	(+12.7)	0.473 ± 0.081	(+16.5)

	Dietary concentration (ppm)	Mean weight ± SD							
		Males (*n* = 5/group)				Females (*n* = 5/group)			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
Liver (g)	0	361.2 ± 29.5	—	2.618 ± 0.283	—	386.3 ± 41.6	—	2.951 ± 0.255	—
	300	347.6 ± 30.9	(−3.8)	2.504 ± 0.265	(−4.4)	303.7* ± 53.5	(−21.4)	2.467 ± 0.472	(−16.4)
	1500	417.0 ± 56.4	(+15.4)	2.883 ± 0.446	(+10.1)	373.6 ± 43.3	(−3.3)	2.962 ± 0.241	(+0.4)
	10 000/7500	497.1** ± 17.5	(+37.6)	3.509** ± 0.001	(+34.0)	452.0 ± 47.6	(+17.0)	3.549** ± 0.15	(+20.3)
Thymus (g)	0	9.404 ± 5.309	—	0.066 ± 0.035	—	11.75 ± 4.758	—	0.088 ± 0.03	—
	300	5.35 ± 2.186	(−43.1)	0.038 ± 0.014	(−42.6)	8.79 ± 2.598	(−25.2)	0.071 ± 0.021	(−19.2)
	1500	7.716 ± 2.739	(−17.9)	0.053 ± 0.017	(−19.9)	9.318 ± 1.919	(−20.7)	0.073 ± 0.009	(−16.9)
	10 000/7500	8.238 ± 3.789	(−12.4)	0.058 ± 0.029	(−12.4)	10.28 ± 4.87	(−12.5)	0.078	(−11.4)
Uterus (g)	0	—	—	—	—	12.37 ± 7.904	—	0.093 ± 0.059	—
	300	—	—	—	—	5.956 ± 5.578	(−51.9)	0.048 ± 0.045	(−48.4)
	1500	—	—	—	—	4.232 ± 1.553	(−65.8)	0.033 ± 0.011	(−64.4)
	7500	—	—	—	—	7.862 ± 7.686	(−36.4)	0.058 ± 0.055	(−37.5)

From Hempel et al. (2009b)
SD, standard deviation; * $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)
[a] Compared with control. Values may not calculate exactly due to rounding.
[b] Percentage of body weight.

The NOAEL was 1500 ppm in both sexes (equal to 45 mg/kg bw per day for males and 51 mg/kg bw per day for females), based on vomiting and clinical chemistry changes seen at 10 000 ppm (equal to 295 mg/kg bw per day) in males and 7500 ppm (equal to 238 mg/kg bw per day) in females (Hempel et al., 2009b).

In a 1-year oral toxicity study, groups of five Beagle dogs of each sex per dose were treated for 52 weeks with fluxapyroxad (purity 99.4%) incorporated into the diet at a concentration of 0, 300, 1500 or 12 000 (males)/9000 (females) ppm (equal to average dose levels of 0, 8, 39 and 335 mg/kg bw per day for males and 0, 9, 43 and 257 mg/kg bw per day for females, respectively). Daily examinations were performed for general observation and mortality. The animals were subjected weekly to a detailed clinical examination and body weight and feed consumption recording. Ophthalmological examinations were performed on all animals predosing and in week 52. Blood samples for haematology and plasma clinical chemistry were withdrawn and urine samples were collected from all animals predosing and in weeks 13, 26 and 52. The animals were sacrificed after 52 weeks of treatment and subjected to necropsy and postmortem examination of major organs and tissues. Organs were weighed, and a full range of tissues was preserved, processed and examined by light microscopy.

There were no deaths at any dose level, and there were no treatment-related effects in the semiquantitative observations made in the home cage or in the open field. Ophthalmological parameters were also unaffected by treatment.

Vomiting was observed in all high-dose animals at the beginning of and midway through the study and was considered adverse (Table 40). Body weight gain was also decreased at the high dose (Table 41). There were no treatment-related adverse effects on haematological parameters (Table 42). In the liver, changes in a number of clinical chemistry parameters at 1500 and/or 12 000/9000 ppm (Table 43) as well as statistically significant changes in absolute and/or relative liver weights at 12 000 ppm in males and 9000 ppm in females were observed (Table 44). The clinical chemistry changes consisted of increased ALP, serum GGT, triglyceride and ALT as well as decreased total protein, albumin, cholesterol, total bilirubin, creatinine, urea and calcium levels in either or both sexes. Histopathological analysis of the organs showed an intracytoplasmic iron deposition in hepatocytes as well as multifocal fibrosis (considered adverse) in the liver at dose levels of 1500 ppm and higher. One high-dose male displayed slight cirrhosis.

Table 40. Clinical observations in dogs administered fluxapyroxad for approximately 12 months

Observation	Males (n = 5/group)				Females (n = 5/group)			
	0 ppm	300 ppm	1500 ppm	12 000 ppm	0 ppm	300 ppm	1500 ppm	9000 ppm
Vomitus	—	—	2	5	1	2	1	5
- on individual animal basis	—	—	#12 (1×) #13 (3×)	#16 (5×) #17 (3×) #18 (2×) #19 (6×) #20 (19×)	#23 (1×)	#28 (1×) #30 (2×)	#33 (1×)	#36 (5×) #37 (4×) #38 (1×) #39 (5×) #40 (1×)
Soft faeces	1	3	2	1	2	1	—	2
Diarrhoea	—	—	—	—	1	—	1	—

From Hempel et al. (2009c)

Table 41. Mean body weight and body weight gain of dogs administered fluxapyroxad for approximately 12 months

	Mean weight ± SD							
	Males (n = 5/group)				Females (n = 5/group)			
	0 ppm	300 ppm	1500 ppm	12 000 ppm	0 ppm	300 ppm	1500 ppm	9000 ppm
Body weight (kg)								
- day 0	12.2 ± 1.9	12.1 ± 1.4	12.5 ± 1.5	12.1 ± 1.4	9.9 ± 1.1	9.8 ± 1.2	10.2 ± 1.9	10.1 ± 0.7
- day 364	16.2 ± 1.2	15.9 ± 1.8	16.9 ± 1.1	14.8 ± 0.7	13.7 ± 0.9	15.0 ± 2.0	15.0 ± 2.0	12.3 ± 1.0
% change[a]	—	*−1.9*	*+4.3*	*−8.6*	—	*+9.5*	*+9.5*	*−10.2*
Overall body weight gain (kg)	4.1 ± 1.7	3.8 ± 1.4	4.4 ± 0.7	2.7 ± 1.6	3.8 ± 0.5	5.2 ± 1.4	4.9 ± 0.6	2.2 ± 1.0
% change[a]	—	*−5.0*	*+8.0*	*−32.5*	—	*+36.8*	*+26.3*	*−42.1**

From Hempel et al. (2009c)

SD, standard deviation; * $P \leq 0.05$ (Dunnett's test, two-sided)

[a] Compared with control.

Table 42. Selected haematology findings of dogs administered fluxapyroxad for 1 year

	Month	Group mean values ± SD							
		Males (n = 5/group)				Females (n = 5/group)			
		0 ppm	300 ppm	1500 ppm	12 000 ppm	0 ppm	300 ppm	1500 ppm	9000 ppm
Leukocytes (%)	−0.5	0.5 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.7 ± 0.3	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.2	0.6 ± 0.1
	3	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.3	0.4 ± 0.3	0.4 ± 0.1	0.2* ± 0.1	0.4 ± 0.1	0.6 ± 0.1
	6	0.3 ± 0.1	0.2 ± 0.0	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.0
	12	0.3 ± 0.1	0.4 ± 0.3	0.3 ± 0.1	0.2 ± 0.1	0.4 ± 0.3	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
Platelets (10^9/l)	−0.5	386 ± 53	476 ± 153	430 ± 55	384 ± 79	395 ± 97	420 ± 84	409 ± 52	398 ± 57
	3	345 ± 34	387 ± 100	395 ± 40	444 ± 136	330 ± 94	305 ± 28	370 ± 25	424 ± 62
	6	334 ± 28	382 ± 83	372 ± 49	508 ± 123	342 ± 59	284 ± 104	410 ± 53	496* ± 101 (+45%)
	12	336 ± 27	386 ± 96	376 ± 45	504** ± 80 (+50%)	371 ± 39	317* ± 22 (−19%)	470** ± 27 (+27%)	480** ± 57 (+29%)
(Activated) partial thromboplastin time (s)	−0.5	11.6 ± 0.5	11.4 ± 0.4	11.5 ± 0.4	11.4 ± 0.4	11.1 ± 0.8	11.8 ± 0.3	11.5 ± 0.5	11.6 ± 0.5
	3	11.7 ± 0.5	11.7 ± 0.5	12.2 ± 0.4	11.5 ± 0.3	11.7 ± 0.5	12.4 ± 0.4	11.6 ± 0.4	11.9 ± 0.7
	6	11.2 ± 0.5	11.4 ± 0.5	11.7 ± 0.3	11.2 ± 0.4	11.4 ± 0.5	12 ± 0.2	11.3 ± 0.6	11.6 ± 0.5
	12	11.2 ± 0.3	11.5 ± 0.4	11.5 ± 0.3	11.5 ± 0.4	11.4 ± 0.5	11.7 ± 0.6	11.1 ± 0.2	11.8 ± 0.6
Prothrombin time (s)	−0.5	7.7 ± 0.6	7.5 ± 0.5	7.6 ± 0.6	7.7 ± 0.5	7.2 ± 0.3	7.5 ± 0.6	7.3 ± 0.3	7.5 ± 0.6
	3	8.0 ± 0.7	7.8 ± 0.5	7.8 ± 0.7	8.4 ± 0.8	7.6 ± 0.3	7.8 ± 0.7	7.5 ± 0.2	7.9 ± 0.6
	6	7.4 ± 0.8	7.4 ± 0.4	7.6 ± 0.7	8.3 ± 0.9	7.4 ± 0.3	7.7 ± 0.9	7.1 ± 0.4	7.7 ± 0.5
	12	7.7 ± 0.9	7.5 ± 0.5	7.4 ± 0.7	8.1 ± 1.0	7.3 ± 0.3	7.2 ± 0.6	6.9 ± 0.3	7.3 ± 0.6
White blood cells (10^9/l)	−0.5	12.92 ± 2.43	12.38 ± 1.30	11.31 ± 0.45	11.50 ± 1.09	12.71 ± 2.16	12.27 ± 1.89	12.18 ± 1.18	12.55 ± 2.50
	3	11.71 ± 1.33	12.91 ± 0.73	11.05 ± 1.44	11.77 ± 3.88	11.89 ± 2.29	12.51 ± 2.11	11.93 ± 1.42	11.14 ± 1.90
	6	11.84 ± 1.86	12.20 ± 0.85	10.19 ± 1.08	11.15 ± 3.79	11.05 ± 2.72	12.97 ± 3.08	11.70 ± 1.98	11.83 ± 2.81
	12	10.56 ± 1.32	12.89* ± 2.15	9.07* ± 0.96	10.45 ± 3.62	9.96 ± 1.21	11.17 ± 1.72	11.95 ± 2.55	10.65 ± 2.40

From Hempel et al. (2009c)

SD, standard deviation; * $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)

Table 43. Selected clinical chemistry findings of dogs administered fluxapyroxad for 1 year

	Month	Group mean values ± SD							
		Males (n = 5/group)				Females (n = 5/group)			
		0 ppm	300 ppm	1500 ppm	12 000 ppm	0 ppm	300 ppm	1500 ppm	9000 ppm
ALP (µkat/l)	−0.5	2.64 ± 0.16	2.30 ± 0.38	2.35 ± 0.46	2.47 ± 0.53	2.76 ± 0.35	2.15* ± 0.33	2.57 ± 0.09	2.46 ± 0.27
	3	1.44 ± 0.18	1.23 ± 0.17	2.07 ± 1.24	17.49** ± 14.19 (+1100%)	1.56 ± 0.16	1.31 ± 0.30	2.17 ± 0.58	8.13** ± 3.85 (+420%)
	6	1.27 ± 0.30	1.27 ± 0.43	2.32 ± 1.25 (+80%)	21.61** ± 15.18 (+1600%)	1.65 ± 0.35	1.22 ± 0.31	3.42 ± 1.27 (+110%)	8.41** ± 4.50 (+410%)
	12	0.96 ± 0.22	0.92 ± 0.18	2.47 ± 2.24 (+160%)	20.54** ± 17.60 (+2000%)	1.35 ± 0.47	1.07 ± 0.38	2.91 ± 1.40 (+120%)	9.64** ± 6.20 (+610%)
GGT (µkat/l)	-0.5	38 ± 8	29 ± 9	29 ± 6	25 ± 8	28 ± 11	40 ± 6	30 ± 13	34 ± 17
	3	28 ± 19	38 ± 13	27 ± 7	55 ± 27	25 ± 8	33 ± 11	35 ± 12	67** ± 18 (+170%)
	6	54 ± 5	49 ± 15	46 ± 12	107* ± 73	53 ± 12	60 ± 11	67 ± 14	91* ± 26 (+72%)
	12	45 ± 10	45 ± 9	33 ± 16	189 ± 184 (+320%)	42 ± 18	51 ± 12	57 ± 19	97* ± 32 (+130%)
ALT (µkat/l)	−0.5	0.46 ± 0.08	0.47 ± 0.13	0.50 ± 0.13	0.55 ± 0.15	0.47 ± 0.11	0.46 ± 0.08	0.47 ± 0.04	0.45 ± 0.08
	3	0.54 ± 0.12	0.82 ± 0.21	0.52 ± 0.08	1.03 ± 0.81	0.54 ± 0.11	0.60 ± 0.16	0.51 ± 0.06	0.47 ± 0.04
	6	0.72 ± 0.13	0.93 ± 0.30	0.58 ± 0.11	1.70 ± 1.52 (+140%)	0.56 ± 0.13	0.65 ± 0.18	0.57 ± 0.12	0.64 ± 0.11
	12	0.71 ± 0.13	1.04 ± 0.31	0.73 ± 0.25	2.31* ± 1.79 (+230%)	0.60 ± 0.19	0.64 ± 0.14	0.55 ± 0.07	0.83 ± 0.27
Protein, total (g/l)	−0.5	60.14 ± 1.63	61.88 ± 2.48	60.31 ± 1.33	61.30 ± 1.58	59.05 ± 0.84	60.37 ± 1.80	59.16 ± 3.07	58.88 ± 1.79
	3	58.52 ± 1.42	58.47 ± 2.37	53.99** ± 1.14 (−7.7%)	49.09 ± 6.00	59.20 ± 1.38	57.16 ± 2.13	55.30 ± 3.09	52.96** ± 2.45 (−11%)
	6	62.13 ± 2.76	61.30 ± 2.09	57.18* ± 1.60 (−8.0%)	51.24* ± 6.13 (−18%)	63.24 ± 1.66	61.21 ± 1.53	57.77** ± 1.72 (−8.6%)	55.11** ± 1.88 (−13%)
	12	61.79 ± 2.37	59.37 ± 2.37	56.42** ± 2.04 (−8.7%)	50.33* ± 5.81 (−19%)	61.78 ± 2.49	60.58 ± 1.44	57.74* ± 0.59 (−6.5%)	52.10** ± 2.71 (−16%)
Albumin (g/l)	−0.5	33.09 ± 1.06	33.60 ± 2.08	33.62 ± 1.04	34.44 ± 0.85	34.16 ± 1.47	34.28 ± 1.59	33.03 ± 1.55	33.53 ± 1.58
	3	34.84 ± 1.25	34.15 ± 1.16	30.68** ± 0.52 (−12%)	26.54** ± 3.48 (−24%)	36.17 ± 1.22	34.51 ± 0.95	32.50** ± 1.56 (−10%)	29.95** ± 1.54 (−17%)

	Month	Group mean values ± SD							
		Males (*n* = 5/group)				Females (*n* = 5/group)			
		0 ppm	300 ppm	1500 ppm	12 000 ppm	0 ppm	300 ppm	1500 ppm	9000 ppm
	6	35.91 ± 0.75	34.75 ± 1.33	31.46** ± 0.90 (−12%)	26.03** ± 3.71 (−28%)	36.77 ± 1.04	35.02 ± 1.30	31.51** ± 0.56 (−14%)	29.88** ± 2.25 (−19%)
	12	36.10 ± 1.08	33.74* ± 1.40 (−6.5%)	31.67** ± 0.76 (−12%)	25.19** ± 3.86 (−30%)	36.40 ± 1.68	34.52 ± 1.39	32.04** ± 0.80 (−12%)	28.14** ± 2.35 (−23%)
Calcium (mmol/l)	−0.5	2.82 ± 0.02	2.82 ± 0.02	2.81 ± 0.04	2.84 ± 0.04	2.89 ± 0.06	2.96 ± 0.05	2.86 ± 0.08	2.90 ± 0.08
	3	2.71 ± 0.03	2.67 ± 0.05	2.59** ± 0.05 (4.4%)	2.51** ± 0.07 (−7.3)	2.76 ± 0.11	2.77 ± 0.06	2.69 ± 0.07	2.65 ± 0.08
	6	2.70 ± 0.07	2.64 ± 0.05	2.60 ± 0.06	2.49* ± 0.07 (−7.8%)	2.77 ± 0.09	2.76 ± 0.05	2.67 ± 0.06	2.69 ± 0.07
	12	2.69 ± 0.06	2.62* ± 0.05 (−2.6%)	2.60* ± 0.04 (−3.3%)	2.49** ± 0.05 (−7.4%)	2.71 ± 0.09	2.70 ± 0.04	2.65 ± 0.05	2.56* ± 0.07 (−5.5%)
Urea (mmol/l)	−0.5	3.96 ± 0.85	4.23 ± 0.93	3.55 ± 0.17	4.07 ± 0.58	3.42 ± 0.39	3.73 ± 0.65	3.74 ± 0.46	3.91 ± 0.67
	3	5.13 ± 0.61	4.82 ± 0.46	4.19 ± 0.31	4.19 ± 0.76	4.87 ± 0.16	4.89 ± 0.57	4.66 ± 0.80	3.72** ± 0.48 (−24%)
	6	5.11 ± 0.64	4.55 ± 0.56	3.95* ± 0.31 (−23%)	3.87* ± 0.75 (−24%)	5.53 ± 1.00	4.87 ± 0.39	5.51 ± 1.00	3.83** ± 0.35 (−31%)
	12	4.88 ± 0.56	4.35 ± 0.51	3.96* ± 0.31 (−19%)	3.44** ± 0.54 (−30%)	4.93 ± 0.86	4.34 ± 0.99	4.37 ± 0.98	3.60 ± 0.64
Creatinine (mmol/l)	−0.5	69.5 ± 3.7	69.9 ± 10.6	72.3 ± 4.6	71.5 ± 5.4	66.6 ± 4.0	66.0 ± 8.3	68.3 ± 6.0	65.6 ± 5.3
	3	85.7 ± 4.2	82.8 ± 7.9	87.4 ± 3.5	79.5 ± 6.0	84.1 ± 4.3	83.9 ± 2.6	84.4 ± 5.5	78.2 ± 6.9
	6	89.7 ± 4.1	85.3 ± 6.5	91.4 ± 2.1	80.0 ± 6.8	84.8 ± 6.7	88.6 ± 5.7	88.1 ± 8.2	79.3 ± 4.4
	12	93.6 ± 7.7	92.2 ± 5.5	92.3 ± 8.1	78.7* ± 7.9 (−16%)	83.6 ± 9.9	88.6 ± 8.0	84.2 ± 7.8	79.2 ± 6.6
Bilirubin, total (μmol/l)	−0.5	2.35 ± 0.30	2.71 ± 0.38	2.57 ± 0.93	2.69 ± 0.10	2.01 ± 0.31	2.77 ± 0.69	2.67 ± 0.44	2.98 ± 1.51
	3	3.61 ± 0.69	3.19 ± 0.88	2.73 ± 0.51	2.82 ± 0.40	4.11 ± 0.69	3.50 ± 1.00	3.10 ± 0.52	2.69** ± 0.38 (−35%)
	6	3.94 ± 1.10	2.86 ± 0.76	2.43* ± 0.46 (−38%)	2.69* ± 0.25 (−32%)	4.19 ± 0.88	3.50 ± 0.60	3.26 ± 0.41	2.06** ± 0.39 (−51%)
	12	4.01 ± 1.35	2.79 ± 0.86	2.54 ± 0.99	2.39 ± 0.23	3.27	2.65	2.16**	1.20**

Table 43 (continued)

	Month	Group mean values ± SD							
		Males (*n* = 5/group)				Females (*n* = 5/group)			
		0 ppm	300 ppm	1500 ppm	12 000 ppm	0 ppm	300 ppm	1500 ppm	9000 ppm
Cholesterol (mmol/l)	−0.5	4.50 ± 0.91	4.26 ± 0.85	4.22 ± 1.06	5.11 ± 1.13	3.77 ± 0.28	3.56 ± 0.51	3.79 ± 0.71	3.52 ± 0.12
	3	3.94 ± 0.25	3.39 ± 0.47	3.20* ± 0.57 (−19%)	2.20** ± 0.78 (−44%)	3.50 ± 0.23	3.18 ± 0.33	3.08 ± 0.48	2.40** ± 0.27 (−31%)
	6	4.08 ± 0.39	3.48 ± 0.45	3.36 ± 0.78	2.51** ± 0.60 (−38%)	4.49 ± 0.78	3.35* ± 0.41	4.11 ± 0.21	2.83** ± 0.32 (−37%)
	12	3.69 ± 0.27	3.12 ± 0.43	3.05 ± 0.63	2.29** ± 0.48 (−38%)	3.83 ± 0.60	3.26 ± 0.69	3.69 ± 0.35	2.24** ± 0.27 (−42%)
Triglycerides (mmol/l)	−0.5	0.31 ± 0.05	0.27 ± 0.06	0.25 ± 0.04	0.29 ± 0.08	0.31 ± 0.06	0.27 ± 0.06	0.31 ± 0.07	0.29 ± 0.08
	3	0.30 ± 0.11	0.26 ± 0.05	0.24 ± 0.06	0.47 ± 0.11	0.30 ± 0.08	0.26 ± 0.08	0.30 ± 0.04	0.34 ± 0.10
	6	0.25 ± 0.06	0.25 ± 0.08	0.22 ± 0.06	0.44** ± 0.08 (+76%)	0.44 ± 0.10	0.29 ± 0.07	0.33 ± 0.12	0.33 ± 0.07
	12	0.28 ± 0.04	0.23 ± 0.08	0.23 ± 0.05	0.44** ± 0.14 (+57%)	0.39 ± 0.15	0.31 ± 0.07	0.34 ± 0.09	0.36 ± 0.05

From Hempel et al. (2009c)
ALP, alkaline phosphatase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; SD, standard deviation; * $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)

Table 44. Selected mean absolute and relative organ weights of dogs administered fluxapyroxad for approximately 12 months

	Dietary concentration (ppm)	Mean weight ± SD							
		Males (*n* = 5/group)				Females (*n* = 5/group)			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
Terminal weight (kg)	0	16.2 ± 1.2	—	—	—	13.84 ± 0.90	—	—	—
	300	15.88 ± 0.87	(−2.0)	—	—	15.04 ± 2.01	(+8.7)	—	—
	1500	16.96 ± 1.07	(+4.7)	—	—	15.22 ± 2.08	(+10.0)	—	—
	12 000/9000	14.82 ± 0.69	(−8.5)	—	—	12.9 ± 1.0	(−6.8)	—	—
Epididymides (g)	0	5.496 ± 0.886	—	0.034 ± 0.006	—	—	—	—	—
	300	4.676 ± 0.353	(−14.9)	0.03 ± 0.003	(−13.5)	—	—	—	—
	1500	4.812 ± 0.192	(−12.4)	0.028 ± 0.002	(−16.6)	—	—	—	—

	Dietary concentration (ppm)	Mean weight ± SD							
		Males (n = 5/group)				Females (n = 5/group)			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
	12 000	4.094 ± 0.544	(−25.5)	0.028 ± 0.003	(−19.2)	—	—	—	—
Heart (g)	0	135.3 ± 12.7	—	0.838 ± 0.083	—	105.3 ± 12.8	—	0.76 ± 0.06	—
	300	132.3 ± 8.2	(−2.2)	0.833 ± 0.035	(−0.5)	111.5 ± 9.3	(+5.8)	0.753 ± 0.127	(−0.9)
	1500	122.9 ± 7.1	(−9.1)	0.726* ± 0.05	(−13.4)	103.6 ± 11.5	(−1.6)	0.684 ± 0.059	(−10.0)
	12 000/9000	122.7 ± 15.5	(−9.3)	0.826 ± 0.071	(−1.4)	100.4 ± 9.1	(−4.7)	0.781 ± 0.085	(+2.8)
Kidneys (g)	0	70.95 ± 3.96	—	0.439 ± 0.024	——	57.20 ± 6.84		0.412 ± 0.026	—
	300	73.74 ± 9.81	(+3.9)	0.464 ± 0.05	(+5.7)	60.88 ± 3.55	(+6.4)	0.411 ± 0.064	(−0.2)
	1500	79.65 ± 6.67	(+12.3)	0.47 ± 0.037	(+7.1)	56.54 ± 7.11	(−1.1)	0.372* ± 0.017	(−9.7)
	12 000/9000	84.90 ± 13.26	(+19.7)	0.571** ± 0.07	(+30.1)	58.18 ± 3.33	(+1.7)	0.453 ± 0.046	(+10.0)
Liver (g)	0	421.9 ± 30.5	—	2.614 ± 0.267	—	375.3 ± 20.0	—	2.723 ± 0.263	—
	300	396.2 ± 39.7	(−6.1)	2.494 ± 0.199	(−4.6)	326.2 ± 51.3	(−13.1)	2.206 ± 0.515	(−19.0)
	1500	432.1 ± 34.1	(+2.4)	2.554 ± 0.23	(−2.3)	395.3 ± 51. 4	(+5.3)	2.614 ± 0.329	(−4.0)
	12 000/9000	524.8** ± 79.6	(+24.4)	3.536** ± 0.46	(+35.3)	431.7* ± 38.6	(+15.0)	3.344** ± 0.076	(+22.8)
Pituitary (g)	0	89.0 ± 13.8	—	0.001 ± 0.0	—	95.4 ± 16.3	—	0.001 ± 0.0	—
	300	97.0 ± 16.6	(+9.0)	0.001 ± 0.0	(+11.5)	82.0 ± 10.7	(−14.0)	0.001 ± 0.0	(−7.1)
	1500	88.0 ± 12.9	(−1.1)	0.001 ± 0.0	(−5.4)	85.6 ± 13.4	(−10.3)	0.001 ± 0.0	(−4.6)
	12 000/9000	93.4 ± 12.3	(+4.9)	0.001* ± 0.0	(+14.8)	87.4 ± 12.74	(−8.4)	0.001 ± 0.0	(+15.0)
Prostate (g)	0	10.64 ± 3.16	—	0.066 ± 0.022	—	—	—	—	—
	300	7.088 ± 2.613	(−33.4)	0.045 ± 0.016	(−32.6)	—	—	—	—
	1500	10.89 ± 1.52	(1.9)	0.064 ± 0.008	(−3.6)	—	—	—	—
	12 000	3.808** ± 0.776	(−64.2)	0.026** ± 0.005	(−60.6)	—	—	—	—
Spleen (g)	0	40.68 ± 3.72	—	0.252 ± 0.028	—	34.2 ± 7.1	—	0.247 ± 0.05	—
	300	35.09 ± 5.89	(−13.7)	0.22 ± 0.03	(−12.7)	29.92 ± 6.43	(−12.5)	0.199 ± 0.034	(−19.4)

Table 44 (continued)

	Dietary concentration (ppm)	Mean weight ± SD							
		Males (n = 5/group)				Females (n = 5/group)			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
	1500	36.26 ± 5.37	(−10.9)	0.214 ± 0.027	(−15.1)	31.05 ± 6.64	(−9.2)	0.204 ± 0.031	(−17.4)
	12 000/9000	28.28* ± 3.94	(−30.5)	0.191* ± 0.031	(−24.2)	24.66 ± 3.49	(−27.9)	0.191 ± 0.022	(−22.7)
Testes (g)	0	26.72 ± 4.71	—	0.166 ± 0.03	—	—	—	—	—
	300	24.76 ± 2.44	(−7.3)	0.156 ± 0.012	(−5.9)	—	—	—	—
	1500	23.71 ± 3.62	(−11.3)	0.14 ± 0.02	(−15.2)	—	—	—	—
	12 000	23.84 ± 1.79	(−10.8)	0.161 ± 0.014	(−2.7)	—	—	—	—

From Hempel et al. (2009c)
SD, standard deviation; * $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)
[a] Compared with control. Values may not calculate exactly due to rounding.
[b] Percentage of body weight.

In the spleen, fine granular iron storage (distinct from haemosiderin) was observed at 1500 and 9000/12 000 ppm (Table 45). This was accompanied by a diffuse atrophy of the red pulp at 9000/12 000 ppm. No histopathological changes were observed in the white pulp. The findings of iron storage and atrophy at the high dose were considered potentially adverse.

Table 45. Incidence of selected macropathological and histopathological lesions in dogs administered fluxapyroxad for 1 year

	Males (n = 5/group)				Females (n = 5/group)			
	0 ppm	300 ppm	1500 ppm	12 000 ppm	0 ppm	300 ppm	1500 ppm	9000 ppm
Macropathology								
Spleen								
No. examined	5	5	5	5	5	5	5	5
Deposition	0	0	1	5	0	0	0	5
Liver								
No. examined	5	5	5	5	5	5	5	5
Discoloration	0	0	0	5	0	0	0	5
Retraction	0	0	0	1	0	0	0	0
Histopathology								
Spleen								
No. examined	5	5	5	5	5	5	5	5
Iron stain, connective tissue	1 [1.0]	0	4 [1.3]	5 [3.4]	0	0	3 [1.0]	5 [3.0]
Atrophy, diffuse, red pulp	0	0	0	4 [2.8]	0	0	0	3 [1.7]

	Males (n = 5/group)				Females (n = 5/group)			
	0 ppm	300 ppm	1500 ppm	12 000 ppm	0 ppm	300 ppm	1500 ppm	9000 ppm
Iron stain, haemosiderin	5	5	5	5	5	5	5	5
	[1.8]	[1.6]	[1.6]	[1.4]	[2.4]	[1.8]	[1.8]	[1.0]
Congestion, (multi)focal	3	2	2	2	2	3	3	1
Hyperplasia, white pulp, nodular	0	0	0	1	0	0	0	0
Liver								
No. examined	5	5	5	5	5	5	5	5
Iron stain, hepatocytes	0	0	4	5	0	0	2	5
			[1.0]	[3.0]			[1.0]	[1.8]
Fibrosis, (multi)focal	0	0	0	4	0	0	2	3
				[2.0]			[1.5]	[1.0]
Cirrhosis, diffuse	0	0	0	1	0	0	0	0
				[2.0]				
Iron stain, Kupffer cells	3	3	4	3	4	3	5	1
	[1.3]	[1.0]	[1.0]	[1.3]	[1.3]	[1.8]	[1.6]	[1.0]
Pigment storage, diffuse	5	2	4	5	2	0	4	5
Infiltration, lymphoid, (multi)focal	2	5	3	5	5	5	3	3
Inflammation, (multi)focal	0	1	0	0	0	0	0	0
Inclusions, intracytoplasm	0	0	1	2	4	3	4	3
Gall bladder								
No. examined	5	5	5	5	5	5	5	5
Pigment storage	0	0	2	2	0	0	4	4
			[1.0]	[1.5]			[1.5]	[1.3]
Prostate								
No. examined	5	5	5	5	—	—	—	—
Atrophy, diffuse	0	0	0	5	—	—	—	—
				[2.6]				
Infiltration, lymphoid, (multi)focal	1	0	2	0	—	—	—	—
Dilatation, glandular	0	1	1	0	—	—	—	—
Testes								
No. examined	5	5	5	5	—	—	—	—
Degeneration, tubular, (multi)focal, unilateral	0	0	3	2	—	—	—	—
			[1.0]	[1.5]				
Multinucleated giant cells	0	2	1	1	—	—	—	—

From Hempel et al. (2009c)

[a] [ ] mean severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked (Grade 4) and massive/severe (Grade 5). The mean severity is the sum of the gradings divided by the incidence.

Changes were also observed in the gall bladder (epithelial storage of granular brown pigment of males and females at 1500 ppm and above, not considered adverse) and the prostate (reduced weight and atrophy without change of the glandular structure at 12 000 ppm, considered adverse).

The NOAEL in females was 300 ppm (equal to 9 mg/kg bw per day), based on hepatic fibrosis and clinical chemistry changes seen at 1500 ppm (equal to 43 mg/kg bw per day). The

NOAEL in males was 1500 ppm (equal to 39 mg/kg bw per day), based on vomiting, clinical chemistry changes and hepatic fibrosis and cirrhosis seen at 12 000 ppm (equal to 335 mg/kg bw per day) (Hempel et al., 2009c).

(b) Dermal application

Rats

In a 28-day dermal toxicity study, groups of 10 Wistar rats of each sex per dose were treated dermally with fluxapyroxad (purity 99.2%) applied to approximately 10% of the body surface area at a dose level of 0, 100, 300 or 1000 mg/kg bw per day for 4 weeks. The volume of application was 4 ml/kg bw, and the duration of treatment was 6 hours daily. The animals were observed twice daily for clinical signs and daily for signs of skin irritation, and a detailed physical examination was performed weekly. Body weights and feed consumption were recorded weekly, and ophthalmoscopic examinations were performed on control and high-dose animals in week 4. Urine samples and blood samples for haematology and plasma chemistry were collected in week 4, and a functional observational battery was performed on all animals. The animals were sacrificed after 4 weeks of treatment and subjected to necropsy and postmortem examination of major organs and tissues. Organs were weighed, and a full range of tissues, including gross lesions, was preserved. Tissue samples from all control and high-dose animals and any gross lesions from all animals were processed and examined by light microscopy.

There were no deaths and no treatment-related general clinical signs or reactions at the dermal application sites at any dose level. There was no effect of treatment on body weight, body weight gain or feed consumption in either sex at any dose level. The haematological and clinical chemistry investigations performed in week 4 did not indicate any treatment-related effects at any dose level. There were no treatment-related gross lesions at necropsy at any dose level. The microscopic examination performed after 4 weeks of treatment did not show treatment-related changes in any of the organs examined in the group treated at 1000 mg/kg bw per day.

An increase in absolute and relative liver weights in both sexes was possibly treatment related (Table 46). As there was neither a histopathological correlate nor any accompanying treatment-related changes in clinical chemistry parameters, this effect was not considered adverse.

The NOAEL in males and females was 1000 mg/kg bw per day, the highest dose tested (Kaspers et al., 2009a).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a carcinogenicity study in mice, fluxapyroxad (purity 99.7% up to study day 349; purity 99.2% from day 350 to end of study) was administered in the diet to 52 C57BL/6 J Rj mice of each sex per dose at a concentration of 0, 150, 750, 3000 or 6000 ppm for 78 weeks (equal to 0, 21, 107, 468 and 996 mg/kg bw per day for males and 0, 33, 158, 652 and 1307 mg/kg bw per day for females, respectively). Clinical signs were recorded at least daily, and a detailed physical examination, including palpation, was performed weekly. Body weights and feed consumption were recorded weekly for 13 weeks and at 4-week intervals thereafter. Blood smears were prepared from all survivors in week 50 and at necropsy and examined for total and differential white blood cell counts at necropsy. All animals were subjected to necropsy, postmortem examination, organ weight recording and tissue preservation. Histopathological evaluation of haematoxylin and eosin (H&E)–stained tissues was performed on all animals from the control and high-dose groups, decedents from all groups and liver, thyroids, gross lesions and lungs (females only) from all groups. In addition, the mandibles of all animals were cut longitudinally into two halves between the incisors. The left half of the mandibles was fixed in 4% formaldehyde solution, and the right half was stored deep-frozen (−20°C), individually. The incisors of mandible and maxilla from all satellite animals were processed histotechnically and evaluated microscopically. Because there were no histopathological findings in satellite animals, the teeth of the animals in the final sacrifice group were not subjected to a

Table 46. Selected mean absolute and relative organ weights of rats after dermal administration of fluxapyroxad for 28 days

	Dietary concentration (ppm)	Mean weight ± SD							
		Males (n = 10/group)				Females (n = 10/group)			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
Terminal weight (g)	0	283.3 ± 18.8				183.3 ± 11.8			
	100	279.1 ± 18.4	(−1.5)			178.1 ± 7.9	(−2.8)		
	300	279.9 ± 19.4	(−1.2)			187.0 ± 7.8	(+2.1)		
	1000	284.5 ± 23.7	(+0.4)			190.8 ± 13.1	(+4.1)		
Liver (mg)	0	6.73 ± 0.49		2.38 ± 0.09		4.70 ± 0.45		2.56 ± 0.14	
	100	6.77 ± 0.56	(+0.6)	2.43 ± 0.12	(+2.1)	4.56 ± 0.41	(−2.9)	2.56 ± 0.16	(−0.1)
	300	7.18 ± 0.99	(+6.6)	2.56 ± 0.20	(+7.5)	4.95 ± 0.31	(+5.2)	2.65 ± 0.19	(+3.3)
	1000	7.70** ± 0.84	(+14.3)	2.70** ± 0.15	(+13.8)	5.35** ± 0.41	(+13.9)	2.81** ± 0.14	(+9.6)
Spleen (mg)	0	0.575 ± 0.083		0.203 ± 0.029		0.413 ± 0.07		0.225 ± 0.03	
	100	0.492* ± 0.064	(−14.4)	0.176 ± 0.022	(−13.2)	0.349* ± 0.027	(−15.5)	0.196* ± 0.014	(−12.7)
	300	0.497* ± 0.073	(−13.6)	0.178 ± 0.026	(−12.4)	0.365 ± 0.039	(−11.6)	0.195* ± 0.021	(−13.1)
	1000	0.505* ± 0.046	(−12.2)	0.178 ± 0.01	(−13.6)	0.417 ± 0.059	(+1.0)	0.219 ± 0.026	(−2.7)

From Kaspers et al. (2009a)

SD, standard deviation; * $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)

[a] Compared with controls. Values may not calculate exactly due to rounding.

[b] Percentage of body weight.

histopathological examination. For detection of iron storage in the femur, a Perl's Prussian Blue stain was performed on six top-dose animals. This examination was performed because iron storage was observed in the femur of rats. No iron storage was detected in the examined bones.

There was no statistically significant effect on mortality in either sex at any dose level. Tooth whitening was observed at and above 3000 ppm in both sexes (Table 47). Systemic toxicity was observed as indicated by effects on body weight development in males at and above 3000 ppm and in females at 6000 ppm (Table 48). The liver was identified as the only target organ, as indicated by changes in clinical chemistry (Table 49) and increases in relative and/or absolute liver weights in males at and above 750 ppm and in females at and above 3000 ppm (Tables 50 and 51). Histopathological examination of the liver revealed mainly periportal/central (males; Zone 3) or peripheral (females; Zone 1) hepatocellular hypertrophy at 6000 ppm and at and above 3000 ppm, respectively (Tables 52 and 53). This effect is not considered adverse. In addition, increased severity and/or incidence of fatty changes in hepatocytes were observed at concentrations of 750 ppm and higher. Discoloration of the mandible and maxilla was observed at and above 3000 ppm in both sexes (Table 54). There were no treatment-related neoplasms (Tables 55 and 56).

Table 47. Selected clinical observations in mice administered fluxapyroxad for about 18 months

	Males					Females				
	0 ppm	150 ppm	750 ppm	3000 ppm	6000 ppm	0 ppm	150 ppm	750 ppm	3000 ppm	6000 ppm
Satellite group										
No. of animals in group	*10*	—	—	—	*10*	*10*	—	—	—	*10*
Tooth whitening, lower jaw	0	—	—	—	10	0	—	—	—	10
Scheduled sacrifice	10	—	—	—	10	10	—	—	—	10
Main (carcinogenicity) group										
No. of animals in group	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*
Tooth whitening, lower jaw	0	0	0	22	50	0	0	0	19	50
Tooth whitening, upper jaw	0	0	0	0	0	0	0	0	1	0
Abdomen, palpable masses	12	16	17	8	5	7	6	4	10	6
Skin, palpable masses	2	5	3	3	2	0	1	0	1	1
Reduced general condition	13	7	14	10	6	11	13	12	14	10
Found dead	4	1	5	5	4	7	3	5	5	1
Sacrificed moribund	5	4	5	5	2	4	5	8	4	2
Scheduled sacrifice	41	45	40	40	44	39	42	37	41	47

From Buesen et al. (2009a)

Table 48. Mean body weights and body weight gain of mice administered fluxapyroxad for about 18 months

	Mean weight ± SD									
	Males					Females				
	0 ppm	150 ppm	750 ppm	3000 ppm	6000 ppm	0 ppm	150 ppm	7500 ppm	3000 ppm	6000 ppm
Satellite group										
No. of animals in group	*10*	—	—	—	*10*	*10*	—	—	—	*10*
Body weight (g)										
- day 0	22.2 ± 1.4	—	—	—	22.0 ± 0.7	19.0 ± 0.8	—	—	—	18.7 ± 0.9
- day 91	32.5 ± 2.1	—	—	—	30.3* ± 1.7 (−6.8%)	25.8 ± 2.7	—	—	—	25.2 ± 1.3
- day 175	36.9 ± 3.1	—	—	—	34.1* ± 1.4 (−7.6%)	30.6 ± 5.2	—	—	—	28.9 ± 3.7
- day 273	41.9 ± 4.3	—	—	—	39.6 ± 3.1	36.1 ± 6.3	—	—	—	31.7 ± 4.4
Cumulative body weight gain (g)										
- day 91	10.3 ± 1.4	—	—	—	8.4** ± 1.5	6.8 ± 2.9	—	—	—	6.6 ± 1.4
% change[a]	—	—	—	—	*−18.8*	—	—	—	—	*−3.4*
- day 175	14.7 ± 2.4	—	—	—	12.2* ± 1.3	11.6 ± 5.4	—	—	—	10.2 ± 3.6
% change[a]	—	—	—	—	*−17.3*	—	—	—	—	*11.8*

	Mean weight ± SD									
	Males					Females				
	0 ppm	150 ppm	750 ppm	3000 ppm	6000 ppm	0 ppm	150 ppm	7500 ppm	3000 ppm	6000 ppm
- day 273	19.8 ± 3.5	—	—	—	17.6 ± 2.8	17.1 ± 6.4	—	—	—	13.0 ± 4.5
% change[a]	—	—	—	—	*−10.8*	—	—	—	—	*−23.9*
Main (carcinogenicity) group										
No. of animals in group[b]	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*
Body weight (g)										
- day 0	22.5 ± 1.0	22.3 ± 1.1	22.5 ± 1.0	22.1 ± 0.9	22.3 ± 1.0	19.1 ± 0.8	18.8 ± 0.8	18.8 ± 1.0	18.7 ± 1.0	18.6* ± 0.7 (−2.6%)
- day 91	33.3 ± 3.1	33.5 ± 2.4	33.7 ± 2.9	31.4** ± 2.6 (−5.7%)	30.5** ± 1.9 (−8.4%)	26.1 ± 2.6	25.8 ± 2.7	25.9 ± 2.8	25.2 ± 2.3	25.1 ± 1.7
- day 175	38.6 ± 4.1	38.8 ± 3.2	39.3 ± 3.8	35.1** ± 3.8 (−9.1%)	33.9** ± 3.0 (−12%)	30.4 ± 4.3	29.4 ± 4.9 (*n* = 49)	30.1 ± 5.3	29.5 ± 4.7	28.6 ± 3.2
- day 371	44.8 ± 5.6	45.2 ± 4.9 (*n* = 48)	45.3 ± 4.6 (*n* = 48)	42.2* ± 4.7 (−7.9%) (*n* = 48)	39.0** ± 3.9 (−13%)	36.4 ± 6.0 (*n* = 49)	36.3 ± 6.5 (*n* = 49)	36.9 ± 6.0 (*n* = 48)	35.5 ± 6.9 (*n* = 50)	34.3 ± 4.8 (*n* = 50)
- day 546	40.5 ± 7.0 (*n* = 41)	43.0 ± 7.8 (*n* = 45)	42.3 ± 7.4 (*n* = 40)	39.7 ± 7.3 (*n* = 40)	**37.5** ± 6.4 (−7.4%) (*n* = 45)	34.9 ± 8.3 (*n* = 40)	34.9 ± 6.1 (*n* = 42)	36.9 ± 7.3 (*n* = 37)	35.7 ± 8.1 (*n* = 41)	36.0 ± 6.6 (*n* = 47)
Cumulative body weight gain (g)										
- day 91	10.8 ± 2.8	11.3 ± 2.1	11.1 ± 2.5	9.3** ± 2.1	8.2** ± 1.7	7.0 ± 2.4	7.0 ± 2.8	7.1 ± 2.5	6.5 ± 2.0	6.5 ± 1.4
% change[a]	—	*4.5*	*3.3*	*−14.0*	*−23.9*	—	*0.8*	*1.9*	*−6.9*	*−7.5*
- day 175	16.1 ± 3.9	16.5 ± 3.0	16.8 ± 3.4	12.9** ± 3.4	11.6** ± 2.6	11.3 ± 4.0	10.6 ± 5.0 (*n* = 49)	11.3 ± 4.8	10.9 ± 4.3	9.9 ± 2.9
% change[a]	—	*2.8*	*4.5*	*−19.6*	*−27.9*	—	*−5.9*	*0.5*	*−3.6*	*−11.6*
- day 371	22.3 ± 5.5	22.9 ± 3.9 (*n* =48)	22.9 ± 4.5 (*n* = 48)	20.1 ± 4.2 (*n* = 48)	16.7** ± 3.8	17.3 ± 5.7 (*n* = 49)	17.6 ± 6.4 (*n* = 49)	18.1 ± 5.7 (*n* = 48)	16.8 ± 6.6	15.7 ± 4.6
% change[a]	—	*3.0*	*2.7*	*−9.6*	*−25.0*	—	*1.8*	*4.7*	*−2.6*	*−9.3*
- day 546	18.1 ± 6.9 (*n* = 41)	20.8 ± 7.6 (*n* = 45)	19.9 ± 7.0 (*n* = 40)	17.6 ± 7.4 (*n* = 40)	15.2 ± 6.2 (*n* = 45)	15.7 ± 8.0 (*n* = 40)	16.1 ± 5.9 (*n* = 42)	18.3 ± 7.1 (*n* = 37)	17.1 ± 8.0 (*n* = 41)	17.3 ± 6.6 (*n* = 47)
% change[a]	—	*14.8*	*9.8*	*−2.7*	*−16.0*	—	*3.0*	*16.5*	*8.8*	*−14.5*

From Buesen et al. (2009a)

SD, standard deviation; * $P \leq 0.05$; ** $P \leq 0.01$ (Dunnett's test, two-sided)

[a] Compared with control.

[b] Unless otherwise noted.

Table 49. Selected clinical chemistry findings in mice administered fluxapyroxad for about 9 months (satellite animals)

	Group mean value ± SD									
	Males (n = 10/group)					Females (n = 10/group)				
	0 ppm	150 ppm	750 ppm	3000 ppm	6000 ppm	0 ppm	150 ppm	750 ppm	3000 ppm	6000 ppm
ALP (µkat/l)	1.07 ± 0.12	—	—	—	1.05 ± 0.10	1.60 ± 0.25	—	—	—	1.95* ± 0.39 (+22%)
Triglycerides (mmol/l)	0.93 ± 0.13	—	—	—	0.60** ± 0.15 (−35%)	0.72 ± 0.22	—	—	—	0.55 ± 0.17
Cholesterol (mmol/l)	2.64 ± 0.39	—	—	—	1.13** ± 0.14 (−57%)	2.31 ± 0.28	—	—	—	1.63** ± 0.30 (−29%)

From Buesen et al. (2009a)
ALP, alkaline phosphatase; SD, standard deviation; * $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)

Table 50. Selected mean absolute and relative) organ weights of mice administered fluxapyroxad for 9 months (interim sacrifice animals)

	Dietary concentration (ppm)	Mean weight ± SD							
		Males (n = 10/group)				Females (n = 10/group)			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
Terminal weight (g)	0	37.78 ± 4.2	—	—	—	31.07 ± 6.38	—	—	—
	6000	34.98 ± 2.8	(−7.4)	—	—	27.72 ± 4.12	(−10.8)	—	—
Adrenal glands (mg)	0	3.5 ± 0.85	—	0.009 ± 0.002	—	9.3 ± 1.57	—	0.031 ± 0.008	—
	6000	5.3** ± 1.4	(+51.4)	0.015** ± 0.004	(+62.5)	9.7 ± 1.57	(+4.3)	0.036 ± 0.005	(+14.4)
Liver (mg)	0	1427.8 ± 213.9	—	3.776 ± 0.312	—	1222.2 ± 104.3	—	4.032 ± 0.58	—
	6000	1934.7** ± 190.4	(35.5)	5.529** ± 0.286	(+46.4)	1502.6** ± 127.3	(+22.9)	5.484 ± 0.56	(+36.0)
Ovaries (mg)	0	—	—	—	—	16.0 ± 2.3	—	0.053 ± 0.012	—
	6000	—	—	—	—	13.9* ± 1.9	(−13.1)	0.051 ± 0.009	(−4.2)

From Buesen et al. (2009a)
SD, standard deviation; * $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two-sided)
[a] Compared with control. Values may not calculate exactly due to rounding.
[b] Percentage of body weight.

Table 51. Selected mean absolute and relative organ weights of mice administered fluxapyroxad for 18 months (carcinogenicity group animals)

	Dietary concentration (ppm)	Mean values ± SD (*n*)							
		Males				Females			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
Terminal weight (g)	0	36.02 ± 6.71 (41)	—	—	—	30.89 ± 7.77 (39)	—	—	—
	150	37.93 ± 7.34 (45)	(+5.3)	—	—	29.93 ± 5.91 (42)	(−3.1)	—	—
	750	37.4 ± 6.9 (40)	(+3.9)	—	—	32.07 ± 6.32 (37)	(+3.8)	—	—
	3000	35.16 ± 6.62 (40)	(−2.4)	—	—	31.16 ± 7.5 (41)	(+0.9)	—	—
	6000	33.67 ± 5.38 (44)	(−6.5)	—	—	31.00 ± 6.22 (47)	(+0.4)	—	—
Adrenal glands (mg)	0	5.17 ± 1.39 (41)	—	0.015 ± 0.006	—	9.026 ± 1.93 (39)	—	0.03 ± 0.008	—
	150	4.82 ± 1.35 (44)	(−6.8)	0.014 ± 0.006	(−3.2)	8.98 ± 1.46 (42)	(−0.5)	0.031 ± 0.009	(+2.8)
	750	4.9 ± 1.08 (40)	(−5.2)	0.014 ± 0.005	(−7.9)	9.24 ± 1.3 (37)	(+2.4)	0.03 ± 0.007	(−2.0)
	3000	5.68 ± 1.72 (40)	(+9.8)	0.017 ± 0.009	(+15.9)	9.34 ± 1.32 (41)	(+3.5)	0.032 ± 0.009	(+4.1)
	6000	5.54 ± 1.28 (44)	(+7.2)	0.017 ± 0.006	(+15.6)	9.26 ± 1.45 (47)	(+2.5)	0.031 ± 0.008	(+2.2)
Kidneys(mg)	0	493.9 ± 40.8 (41)	—	1.42 ± 0.29	—	446.5 ± 45.9(39)	—	1.52 ± 0.34	—
	150	510.6 ± 58.6 (45)	(+3.4)	1.38 ± 0.25	(−2.4)	439.5 ± 107.2 (42)	(−1.6)	1.51 ± 0.4	(−0.7)
	750	493.8 ± 81.6 (40)	(0.0)	1.36 ± 0.29	(−4.3)	429.2 ± 35.7 (37)	(−3.9)	1.4 ± 0.3	(−8.7)
	3000	458.1** ± 43.4 (40)	(−7.3)	1.34 ± 0.24	(−5.6)	431.0 ± 52.4 (41)	(−3.5)	1.4 ± 0.31	(−5.1)
	6000	473.3* ± 42.2 (44)	(−4.2)	1.44 ± 0.24	(+1.2)	438.5 ± 54.3 (47)	(−1.8)	1.46 ± 0.27	(−4.3)
Liver (mg)	0	1424.7 ± 269.3 (41)	—	4.04 ± 0.82	—	1443.7 ± 247.97 (39)	—	4.89 ± 1.202	—
	150	1635.7* ± 531.6 (45)	(+14.8)	4.47 ± 1.87	(+10.7)	1365.8 ± 131.37 (42)	(−5.4)	4.7 ± 0.79	(−3.9)
	750	1619.9** ± 400.8 (40)	(+13.7)	4.44* ± 1.4*	(+9.9)	1412.6 ± 145.5 (37)	(−2.2)	4.55 ± 0.87	(−6.9)
	3000	1742.4** ± 581.6 (40)	(+22.3)	5.07* ± 2.06*	(+25.6)	1574.0** ± 230.15 (41)	(+9.0)	5.22 ± 0.96	(+6.9)
	6000	1761.0** ± 244.6 (44)	(+23.6)	5.31** ± 0.9	(+31.6)	1706.0** ± 178.9 (47)	(+18.2)	5.65** ± 0.85	(+15.5)
Spleen (mg)	0	116.0 ± 82.1 (41)	—	0.36 ± 0.32	—	203.5 ± 226.7 (39)	—	0.76 ± 0.95	—
	150	134.8 ± 237.3 (45)	(+16.2)	0.418 ± 0.83	(+16.8)	163.1 ± 83.9 (42)	(−19.8)	0.59 ± 0.39	(−22.4)

Table 51 (continued)

Dietary concentration (ppm)	Mean values ± SD (*n*)							
	Males				Females			
	Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
750	102.4 ± 72.0 (40)	(−11.7)	0.31 ± 0.3	(−13.1)	131.7 ± 89.8 (37)	(−35.3)	0.46 ± 0.43	(−40.0)
3000	86.75* ± 58.6 (40)	(−25.2)	0.28 ± 0.27	(−21.8)	180.8 ± 235.6 (41)	(−11.2)	0.69 ± 1.16	(−8.7)
6000	85.61* ± 60.9 (44)	(−26.2)	0.28 ± 0.26	(−22.1)	189.6 ± 379.7 (47)	(−6.9)	0.77 ± 2.03	(+1.8)

From Buesen et al. (2009a)

SD, standard deviation; * $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)

[a] Compared with control. Values may not calculate exactly due to rounding.

[b] Percentage of body weight.

Table 52. Incidence of selected histopathological lesions in mice administered fluxapyroxad for 9 months (interim sacrifice animals)

	Males					Females				
	0 ppm	150 ppm	750 ppm	3000 ppm	6000 ppm	0 ppm	150 ppm	750 ppm	3000 ppm	6000 ppm
Liver										
No. examined	*10*	—	—	—	*10*	*10*	—	—	—	*10*
Fatty change	10	—	—	—	10	10	—	—	—	10
	[2.3][a]				[3.4]	[1.7]				[1.4]
- macrovesicular	2	—	—	—	10	0	—	—	—	2
Hypertrophy, peripheral	0	—	—	—	0	0	—	—	—	3
										[1.0]

From Buesen et al. (2009a)

[a] [ ] mean severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked (Grade 4) and massive/severe (Grade 5). The mean severity is the sum of the gradings divided by the incidence of the respective finding.

Table 53. Incidence of selected histopathological lesions in mice administered fluxapyroxad for 18 months (carcinogenicity group)

	Males					Females				
	0 ppm	150 ppm	750 ppm	3000 ppm	6000 ppm	0 ppm	150 ppm	750 ppm	3000 ppm	6000 ppm
Liver										
No. examined	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*
Hypertrophy, central	4	2	2	2	24**	0	0	0	2	0
	[1.0][a]	[1.0]	[1.0]	[1.5]	[1.5]				[2.0]	
Hypertrophy, peripheral	2	1	2	6	5	6	5	11	15*	47**
	[1.0]	[1.0]	[1.0]	[1.7]	[1.4]	[1.3]	[1.0]	[1.1]	[1.3]	[1.7]
Fatty change	36	40	40	40	42	33	39	41	32	41
	[2.0]	[2.1]	[2.4]	[2.6]	[2.6]	[2.3]	[2.2]	[2.3]	[2.8]	[2.4]
- macrovesicular	17	22	30**	36**	36**	14	12	26*	26*	29*

From Buesen et al. (2009a)
* $P \leq 0.05$; ** $P \leq 0.01$ (Fisher's exact test, one-sided)
[a] [ ] mean severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked (Grade 4) and massive/severe (Grade 5). The mean severity is the sum of the gradings divided by the incidence of the respective finding.

Table 54. Incidence of selected gross pathological findings in mice administered fluxapyroxad for 18 months (carcinogenicity group animals)

	Males					Females				
	0 ppm	150 ppm	750 ppm	3000 ppm	6000 ppm	0 ppm	150 ppm	750 ppm	3000 ppm	6000 ppm
Incisor										
No. examined	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*
Mandible, discoloration	0	0	0	23	48	0	0	2	17	49
Maxilla, discoloration	0	0	0	9	43	0	0	1	11	46
Liver										
No. examined	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*
Cyst	1	0	0	0	0	0	0	0	0	0
Deformation	0	0	0	0	0	0	0	0	0	1
Discoloration	2	1	1	2	2	0	0	1	0	1
Enlarged	0	3	3	3	9	5	1	0	3	4
Focus	10	9	15	9	19	10	9	10	13	4
Granular surface	0	0	0	0	0	0	0	0	1	1
Mass	3	4	2	6	5	4	1	0	2	2
Necrosis	0	1	0	0	0	1	0	0	1	0
Organ size reduced	1	0	0	0	0	0	0	0	0	0
Prominent acinar pattern	0	0	1	1	1	0	1	1	2	0
Torsion	0	1	0	0	0	0	0	1	0	0

From Buesen et al. (2009a)

Table 55. Incidence of select primary neoplastic findings[a] in mice administered fluxapyroxad for 18 months

	Males					Females				
	0 ppm	150 ppm	750 ppm	3000 ppm	6000 ppm	0 ppm	150 ppm	750 ppm	3000 ppm	6000 ppm
Epididymides										
No. examined	*50*	*13*	*24*	*14*	*50*	—	—	—	—	—
Sarcoma, not otherwise specified	0	0	1	0	0	—	—	—	—	—
Eyes with optic nerve										
No. examined	*50*	*5*	*13*	*11*	*50*	*50*	*9*	*16*	*12*	*50*
Sarcoma, not otherwise specified	0	0	0	0	1	0	0	0	0	0
Forestomach										
No. examined	*50*	*6*	*11*	*12*	*50*	*50*	*8*	*13*	*9*	*50*
Papilloma, squamous	0	1	0	0	0	0	0	0	0	0

Table 55 (continued)

	Males					Females				
	0 ppm	150 ppm	750 ppm	3000 ppm	6000 ppm	0 ppm	150 ppm	750 ppm	3000 ppm	6000 ppm
Glandular stomach										
No. examined	*50*	*8*	*11*	*11*	*50*	*50*	*14*	*16*	*17*	*50*
Adenoma	0	0	0	0	0	0	0	0	1	0
Adenocarcinoma	0	0	1	0	0	0	0	0	0	0
Harderian glands										
No. examined	*49*	*5*	*11*	*11*	*50*	*50*	*8*	*15*	*10*	*49*
Adenoma	2	0	0	0	2	2	0	1	1	0
Haemolymphoreticular system										
No. examined	*50*	*23*	*16*	*17*	*50*	*50*	*18*	*21*	*21*	*50*
Lymphoma, malignant	11	13	5	10	4	4	11	9	11	7
Sarcoma, histiocytic	4	4	6	2	4	8	4	3	6	7
Jejunum										
No. examined	*49*	*13*	*17*	*20*	*50*	*50*	*14*	*20*	*20*	*50*
Adenocarcinoma	2	0	1	0	1	1	0	0	1	0
Kidneys										
No. examined	*50*	*6*	*12*	*10*	*50*	*50*	*10*	*14*	*10*	*50*
Papilloma, transitional cell	1	0	0	0	0	0	0	0	0	0
Liver										
No. examined	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*
Adenoma, hepatocellular	0	3	1	1	2	0	0	0	2	3
Carcinoma, hepatocellular	1	3	1	3	3	1	0	0	0	0
Combined hepatocellular tumours	1	5[b]	2	4	5	1	0	0	2	3
Haemangioma	0	0	1	0	0	1	0	0	0	0
Haemangiosarcoma	0	0	0	1	0	0	0	0	0	0
Lungs										
No. examined	*50*	*9*	*11*	*12*	*50*	*50*	*9*	*14*	*9*	*50*
Adenoma, bronchioloalveolar	0	2	1	2	0	1	1	0	0	1
Carcinoma, bronchioloalveolar	2	0	0	0	0	0	0	1	0	0
Parotid glands										
No. examined	0	0	0	1	0	0	0	0	0	0
Adenocarcinoma	0	0	0	1	0	0	0	0	0	0
Pituitary gland										
No. examined	*50*	*6*	*10*	*10*	*49*	*49*	*15*	*20*	*18*	*49*
Adenoma, pars distalis	1	0	0	0	0	11	7	6	12	17
Skin										
No. examined	*50*	*14*	*17*	*16*	*50*	*50*	*16*	*21*	*17*	*50*
Fibrosarcoma	0	1	0	0	0	0	0	0	1	0
Spleen										
No. examined	*49*	*11*	*12*	*14*	*50*	*50*	*22*	*20*	*21*	*49*
Haemangiosarcoma	1	0	0	0	0	0	0	0	0	0

	Males					Females				
	0 ppm	150 ppm	750 ppm	3000 ppm	6000 ppm	0 ppm	150 ppm	750 ppm	3000 ppm	6000 ppm
Thyroid glands										
No. examined	*48*	*50*	*50*	*50*	*50*	*50*	*48*	*49*	*50*	*50*
Adenoma, follicular cell	0	0	0	0	0	2	0	0	0	2
Uterus										
No. examined	—	—	—	—	—	*50*	*38*	*39*	*40*	*50*
Sarcoma, endometrial stromal	—	—	—	—	—	0	0	0	0	1
Leiomyosarcoma	—	—	—	—	—	0	0	0	1	0

From Buesen et al. (2009a)

[a] This table does not list the incidences of infiltrates of systemic lymphoma and histiocytic sarcoma as well as of metastases in the various organs.

[b] Low-dose male #74 had both an adenoma and a carcinoma.

Table 56. Historical control incidence of hepatocellular tumours in female C57BL mice

Study	Supplier	Study duration (month/year)	No. of animals	Adenoma	(%)	Carcinoma	(%)	Combined	(%)
88082	CRD	8/1989–2/1991	100	0	0	1	1	1	1
88112	IF	11/1990–5/1992	100	0	0	0	0	0	0
90128	CRD	6/1992–1/1994	50	0	0	1	2	1	2
91028	CRD	10/1991–4/1993	50	0	0	4	8	4	8
92024	CRD	10/1992–4/1994	50	3	6	3	6	5	10
92070	CRUSA	9/1993–3/1995	50	0	0	0	0	0	0
94012	CRUSA	10/1994–4/1996	50	2	4	0	0	2	4
97103	Janvier	2/1998–8/1999	50	0	0	0	0	0	0
98106	Janvier	11/1999–5/2001	50	2	4	1	2	3	6
98112	Janvier	11/1999–5/2901	50	1	2	0	0	1	2
01151	CRD	8/2004–2/2006	50	3	6	1	2	4	8
01177	CRD	5/2005–11/2006	50	1	2	1	2	2	4
04090	Janvier	9/2006–3/2008	50	2	4	0	0	2	4
Sum			750	14	—	12	—	26	—
Mean			—	—	1.9	—	1.6	—	3.5
Number of studies			13	—	—	—	—	—	—
Maximum			—	—	6	—	8	—	10
Minimum			—	—	0	—	0	—	0

From Buesen et al. (2009a)

CRD, Charles River Germany; CRUSA, Charles River USA; IF, Iffa Credo, France

The NOAEL for chronic toxicity was 750 ppm (equal to 107 mg/kg bw per day for males and 158 mg/kg bw per day for females), based on decreased body weight seen at 3000 ppm (equal to 468 mg/kg bw per day for males and 652 mg/kg bw per day for females) (Buesen et al., 2009a).

Rats

In a carcinogenicity study in rats, fluxapyroxad (purity 99.7% up to day 454 and 99.2% thereafter) was administered in the diet to 50 Wistar rats of each sex per dose at a concentration of 0,

50, 250, 1500 or 3000 ppm (equal to 0, 2.1, 11, 68 and 145 mg/kg bw per day for males and 0, 2.7, 14, 82 and 182 mg/kg bw per day for females, respectively) for 104 weeks. A satellite group of 10 animals of each sex per dose was sacrificed at 1 year to assess chronic toxicity. The animals were observed twice daily for viability, clinical signs were recorded daily, and a detailed physical examination, including palpation for masses, was performed weekly. Body weights and feed consumption were recorded weekly for 13 weeks and every 4 weeks thereafter. Blood samples from all surviving animals at weeks 52, 79 and 105 were analysed for total red and white blood cell counts and differential white blood cell counts. All animals, including decedents, were subjected to necropsy, postmortem examination, organ weight recording and tissue preservation. Major organs and tissues from the control and high-dose groups, premature decedents from all groups, gross lesions from all animals and adrenals, liver, lungs, kidneys, ovaries and thyroid glands from all animals were processed and examined by light microscopy. From all satellite animals, the visible, rostral part of the incisors was cut off during necropsy and stored deep-frozen (20 °C). The remaining part of the incisors was fixed with the respective bone (mandible, maxilla) in 4% formaldehyde solution, processed histotechnically and evaluated by light microscopy. In addition, the mandible was cut longitudinally into two halves in the midline between the incisors from all main group animals sacrificed at study termination. The left half of the mandible was fixed in 4% formaldehyde solution, and the right half was stored deep-frozen at 20 °C.

Besides standard H&E staining, the following samples were stained with Perl's Prussian Blue stain (PPB) for detection of ferric iron (Fe^{3+}) and evaluated by light microscopy:

- Liver: all main group males of control and top dose;
- Liver: all female groups;
- Liver: single satellite group animals of the top dose (males #295 and #298 and females #593 and #594). In addition, an autofluorescence investigation was performed with PPB-stained liver sample of the single satellite animals for the detection of lipofuscin;
- Femur with knee joint: all animals of all test groups;
- Bulla tympanica and/or parietal bone (dorsal skull): control male #1; top-dose male #243;
- Frontal bone: top-dose females #560 and #565. Additionally, the modified Kossa stain for the detection of calcium carbonate and calcium phosphate was performed. These tissues were also treated with Kardasewitsch solution to remove possible formalin pigment followed by routine H&E stain;
- Nasal cavity: control males #13 and #19, top-dose males #247, #257 and #273, control female #307 and top-dose female #541;
- Mandibular incisors: two female satellite animals, control group and the 1500 ppm group.

For detection of total iron (ferrous [Fe^{2+}] and ferric [Fe^{3+}] iron), a Turnbull stain was performed on the femur of one control male (#1) and one top-dose male (#273).

Immunohistochemical investigations were performed on different tissue sections of several animals. These included:

- Pituitary gland: adrenocorticotropic hormone–specific antibodies (control male #45, low-dose male #89, low-intermediate-dose males #140 and #166, high-intermediate-dose male #199, top-dose males #257 and #285), low-dose female #373;
- Pituitary gland: pan-Cytokeratin, Vimentin, S-100, neuron-specific enolase and glial fibrillary acidic protein (all stains: low-intermediate-dose male #140);
- Thymus: Vimentin and pan-Cytokeratin (control male #28);
- Epididymis: Vimentin, pan-Cytokeratin and Factor 8 (control male #32);
- Brain: pan-Cytokeratin, Vimentin, neurofilament, S-100 and glial fibrillary acidic protein (top-dose satellite male #297).

Periodic acid Schiff stains were performed on the brain and on the cerebellar gross lesion of top-dose male #259 and on the femur of control male #39 and top-dose male #273. Additionally, Grocott stain was performed on the femur of the latter two males. Data were analysed statistically where appropriate.

There was no treatment-related effect at any dose level on the survival of either sex. Treatment-related clinical signs were limited to tooth whitening in both sexes at and above 1500 ppm (Table 57). Body weight decreases of 8% after 2 years were noted in males at 3000 ppm (Table 58). In females, body weights were decreased 11.9% or more at and above 250 ppm. This indicated that dosing was adequate. There was no effect on feed consumption (Table 59). Haematological changes were mainly restricted to female rats and consisted of slightly decreased haemoglobin, mean corpuscular volume and mean corpuscular haemoglobin concentrations at 1500 ppm and/or 3000 ppm (Table 60). None of these effects was considered adverse. However, shorter prothrombin times were recorded for females at dose levels of 250 ppm and higher and in males at 1500 ppm and higher, which were considered potentially adverse due to possible hypercoagulability. Changes in several clinical chemistry parameters were also observed (Table 61). These consisted of increased serum GGT (considered equivocal) in males and/or females at and above 1500 ppm, increased albumin and globulin in males and/or females at and above 1500 ppm (not considered adverse), increased cholesterol at 1500 ppm and above and increased triglyceride levels in females at 3000 ppm (considered potentially adverse), decreased glucose in males at and above 250 ppm (not to a magnitude considered adverse) and decreased total bilirubin in males and females at and above 250 ppm (not considered adverse).

Table 57. Selected clinical observations in rats administered fluxapyroxad for about 24 months (chronic toxicity and carcinogenicity groups)

	Males (n = 60/group)					Females (n = 60/group)				
	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm
Tooth whitening, lower jaw	0	0	0	17	9	0	0	0	24	3
Tooth whitening, upper jaw	0	0	0	0	1	0	0	0	0	0
Abdomen, palpable masses	2	2	1	1	1	3	1	4	7	11
Skin, palpable masses	6	8	10	9	6	15	16	17	5	10
Reduced general condition	4	8	4	2	8	6	6	8	10	9
Found dead (chronic toxicity group)	0	0	0	0	1	0	0	0	0	0
Found dead (carcinogenicity group)	4	3	2	4	7	7	3	1	7	6
Sacrificed moribund (chronic toxicity group)	0	0	0	0	0	0	0	0	0	0
Sacrificed moribund (carcinogenicity group)	2	5	4	1	3	6	7	8	8	5
Scheduled sacrifice (chronic toxicity group)	10	10	10	10	9	10	10	10	10	10
Scheduled sacrifice (carcinogenicity group)	44	42	44	45	41	37	40	41	35	39

From Buesen et al. (2009b)

Table 58. Mean body weight and body weight gain of rats administered fluxapyroxad for up to 2 years (chronic toxicity and carcinogenicity groups)

	Mean values ± SD (n)									
	Males					Females				
	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm
Body weight (g)										
Day 0	176.9 ± 8.7 (60)	176.2 ± 9.2 (60)	176.6 ± 10.0 (60)	174.9 ± 9.3 (60)	173.3 ± 8.6 (60)	134.8 ± 8.7 (60)	135.5 ± 7.2 (60)	134.3 ± 6.6 (60)	133.2 ± 6.9 (60)	132.5 ± 7.6 (60)

Table 58 (continued)

	Mean values ± SD (*n*)									
	Males					Females				
	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm
Day 91	394.1 ± 34.8 (60)	400.7 ± 33.6 (60)	397.0 ± 39.1 (60)	392.7 ± 29.9 (60)	373.0** ± 29.0 (60)	239.5 ± 18.4 (60)	236.7 ± 16.2 (60)	234.3 ± 15.3 (60)	228.0** ± 13.7 (60)	224.1** ± 15.1 (60)
% change[a]	—	*+1.7*	*+0.8*	*−0.3*	*−5.4*	—	*−1.2*	*−2.1*	*−4.8*	*−6.4*
Day 371	496.9 ± 56.0 (50)	510.2 ± 57.2 (49)	506.2 ± 54.2 (49)	497.5 ± 38.1 (50)	467.2** ± 45.6 (49)	284.4 ± 30.5 (49)	276.4 ± 26.5 (50)	268.7** ± 21.0 (50)	264.6** ± 21.3 (50)	253.3** ± 19.0 (50)
% change[a]	—	*+2.7*	*+1.9*	*+0.1*	*−6.0*	—	*−2.8*	*−5.5*	*−7.0*	*−10.9*
Day 728	557.6 ± 67.2 (45)	576.9 ± 85.7 (42)	567.7 ± 66.8 (44)	539.1 ± 62.0 (45)	502.2** ± 56.1 (41)	359.8 ± 51.8 (37)	343.1 ± 50.0 (41)	317.1** ± 39.3 (41)	301.3** ± 29.6 (37)	277.7** ± 37.3 (40)
% change[a]	—	*+6.3*	*−1.0*	*+0.6*	*−8.4*	—	*−4.7*	*−11.9*	*−16.3*	*−22.8*
Overall body weight gain (g)										
Day 91	217.2 ± 32.2 (60)	224.5 ± 30.8 (60)	220.4 ± 34.2 (60)	217.8 ± 27.1 (60)	199.7** ± 25.7 (60)	104.7 ± 13.8 (60)	101.2 ± 12.6 (60)	100.0 ± 12.1 (60)	94.8** ± 11.9 (60)	91.6** ± 11.4 (60)
% change[a]	—	*+3.4*	*+1.5*	*+0.3*	*−8.1*	—	*−3.3*	*−4.5*	*−9.4*	*−12.5*
Day 371	320.0 ± 54.5 (50)	333.3 ± 54.9 (49)	329.0 ± 50.8 (49)	322.7 ± 35.7 (50)	293.2* ± 42.9 (49)	149.4 ± 27.2 (49)	140.5 ± 24.4 (50)	134.2** ± 19.8 (50)	131.4** ± 20.4 (50)	120.6** ± 15.1 (50)
% change[a]	—	*+4.1*	*+2.8*	*+0.8*	*−8.4*	—	*−5.9*	*−10.2*	*−12.0*	*−19.2*
Day 728	380.9 ± 65.9 (45)	400.7 ± 85.1 (42)	390.1 ± 64.1 (44)	365.4 ± 59.4 (45)	329.8** ± 54.7 (41)	224.2 ± 51.6 (37)	206.7 ± 47.1 (41)	182.8** ± 38.7 (41)	167.9** ± 28.3 (37)	144.9** ± 34.7 (40)
% change[a]	—	*+5.2*	*+2.4*	*−4.1*	*−13.4*	—	*−7.8*	*−18.5*	*−25.1*	*−35.4*

From Buesen et al. (2009b)

SD, standard deviation; * $P \leq 0.05$; ** $P \leq 0.01$ (Dunnett's test, two-sided)

[a] Compared with control.

Table 59. Cumulative feed consumption of rats administered fluxapyroxad for 2 years

	Cumulative feed consumption (g/animal)									
	Males					Females				
	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm
Days 0–371[a]	6944	7218	7594	7568	7711	10 522	10 557	10 396	10 135	107 61
% change[a]	—	*+3.9*	*+9.4*	*+9.0*	*+11.0*	—	*+0.3*	*−1.2*	*−3.7*	*+2.3*
Days 0–728	13 633	14 552	15 141	14 767	15 082	5039	5084	5074	4849	5161
% change[a]	—	*+6.7*	*+11.1*	*+8.3*	*+10.6*	—	*+0.9*	*+0.7*	*−3.8*	*+2.4*

From Buesen et al. (2009b)

[a] Compared with control. Values were calculated based on mean individual daily feed. Values may not calculate exactly due to rounding of mean values.

Table 60. Selected haematology findings in rats administered fluxapyroxad for 1 year

Dietary concentration (ppm)	Study day	Mean values ± SD								
		RBC (10^{12}/l)	HGB (mmol/l)	HCT (1/1)	MCV (fl)	MCH (fmol)	MCHC (mmol/l)	HQT (s)	NEUTA (10^9/l)	EOS (%)
Males (*n* = 10)										
0 (control)	92	8.78 ± 0.44	9.2 ± 0.3	0.419 ± 0.015	47.7 ± 1.5	1.05 ± 0.04	22.03 ± 0.33	35.1 ± 2.4	0.90 ± 0.13	2.3 ± 0.7
	183	8.48 ± 0.42	9.4 ± 0.2	0.407 ± 0.015	48.0 ± 1.5	1.12 ± 0.06	23.22 ± 0.65	34.4 ± 2.7	1.01 ± 0.13	2.5 ± 0.7
	365	8.47 ± 0.38	9.4 ± 0.2	0.414 ± 0.012	48.9 ± 1.9	1.12 ± 0.05	22.82 ± 0.38	34.1 ± 1.5	1.07 ± 0.28	2.7 ± 0.6
50	92	9.00 ± 0.23	9.3 ± 0.2	0.422 ± 0.012	47.0 ± 1.0	1.03 ± 0.03	21.94 ± 0.28	35.0 ± 2.0	1.10** ± 0.17 (+22%)	2.9 ± 0.9
	183	8.66 ± 0.35	9.4 ± 0.4	0.406 ± 0.018	46.9 ± 1.1	1.09 ± 0.03	23.25 ± 0.40	34.6 ± 2.0	1.11 ± 0.19	2.7 ± 0.4
	365	8.55 ± 0.25	9.3 ± 0.2	0.407 ± 0.014	47.6 ± 1.2	1.09 ± 0.03	22.80 ± 0.48	33.9 ± 1.9	1.24 ± 0.27	3.3* ± 0.6 (+22%)
250	92	8.82 ± 0.35	9.4 ± 0.4	0.418 ± 0.017	47.4 ± 1.3	1.07 ± 0.04	22.47* ± 0.26 (+2.0%)	34.0 ± 2.3	1.14** ± 0.20 (+27%)	2.3 ± 0.7
	183	8.47 ± 0.57	9.6 ± 0.4	0.404 ± 0.023	47.8 ± 1.5	1.14 ± 0.07	23.86 ± 0.98	34.3 ± 3.0	1.09 ± 0.25	2.4 ± 0.8
	365	8.48 ± 0.33	9.4 ± 0.4	0.408 ± 0.016	48.2 ± 1.3	1.11 ± 0.03	23.08 ± 0.28	33.8 ± 3.0	1.52 ± 0.88	2.6 ± 0.7
1500	92	8.74 ± 0.29	9.3 ± 0.3	0.419 ± 0.016	47.9 ± 1.4	1.07 ± 0.04	22.27 ± 0.31	33.8 ± 1.8	1.01 ± 0.26	2.4 ± 1.1
	183	8.38 ± 0.52	9.5 ± 0.1	0.402 ± 0.005	48.0 ± 1.7	1.13 ± 0.05	23.55 ± 0.38	33.0 ± 1.5	1.04 ± 0.23	2.4 ± 0.7
	365	8.26 ± 0.31	9.2 ± 0.2	0.394** ± 0.006 (−4.8%)	47.8 ± 1.6	1.11 ± 0.05	23.24 ± 0.40	32.4* ± 1.8 (−1.7 s)	1.16 ± 0.19	2.7 ± 1.1
3000	92	8.66 ± 0.52	9.3 ± 0.4	0.419 ± 0.016	48.4 ± 2.0	1.08 ± 0.07	22.26 ± 0.44	32.2** ± 2.2 (−2.9 s)	1.09 ± 0.40	1.9 ± 0.7
	183	8.35 ± 0.50	9.6 ± 0.2	0.407 ± 0.015	48.8 ± 1.8	1.15 ± 0.07	23.48 ± 0.71	32.0 ± 3.2 (−3.1 s)	1.07 ± 0.19	1.9 ± 0.7
	365	8.29 ± 0.30	9.1 ± 0.4	0.398* ± 0.012 (−3.9%)	48.0 ± 1.4	1.11 ± 0.06	23.03 ± 0.74	31.3** ± 2.0 (−2.8 s)	1.32 ± 0.43	2.3 ± 0.4
Females (*n* = 10)										
0 (control)	96	7.88 ± 0.43	8.8 ± 0.2	0.394 ± 0.011	50.1 ± 1.7	1.12 ± 0.05	22.34 ± 0.32	32.3 ± 0.9	0.65 ± 0.18	2.6 ± 0.9
	185	7.48 ± 0.43	9.1 ± 0.2	0.378 ± 0.018	50.6 ± 1.7	1.22 ± 0.07	24.18 ± 0.97	32.3 ± 0.9	0.68 ± 0.28	2.5 ± 0.9
	367	7.63 ± 0.41	9.2 ± 0.3	0.394 ± 0.015	51.7 ± 1.5	1.21 ± 0.05	23.34 ± 0.44	31.4 ± 1.3	0.82 ± 0.30	2.4 ± 0.9

Table 60 (continued)

Dietary concentration (ppm)	Study day	Mean values ± SD								
		RBC (10^{12}/l)	HGB (mmol/l)	HCT (1/1)	MCV (fl)	MCH (fmol)	MCHC (mmol/l)	HQT (s)	NEUTA (10^9/l)	EOS (%)
50	96	8.01 ± 0.42	8.9 ± 0.2	0.399 ± 0.011	49.8 ± 1.3	1.12 ± 0.04	22.43 ± 0.41	31.8 ± 1.8	0.62 ± 0.16	1.8* ± 0.7 (−31%)
	185	7.43 ± 0.29	9.0 ± 0.2	0.373 ± 0.012	50.3 ± 1.3	1.21 ± 0.06	24.06 ± 0.89	31.8 ± 1.1	0.78 ± 0.38	2.1 ± 0.4
	367	7.58 ± 0.31	9.0 ± 0.4	0.390 ± 0.018	51.4 ± 1.4	1.19 ± 0.04	23.12 ± 0.32	31.3 ± 2.1	0.82 ± 0.33	2.1 ± 0.7
250	96	7.89 ± 0.41	8.9 ± 0.4	0.400 ± 0.016	50.8 ± 1.7	1.13 ± 0.05	22.21 ± 0.36	31.5 ± 1.4	0.75 ± 0.28	2.3 ± 1.1
	185	7.50 ± 0.45	9.1 ± 0.4	0.380 ± 0.017	50.8 ± 1.8	1.22 ± 0.07	23.95 ± 0.71	30.7* ± 1.7	0.88 ± 0.20	2.7 ± 0.8
	367	7.53 ± 0.36	9.1 ± 0.3	0.394 ± 0.011	52.4 ± 1.9	1.21 ± 0.08	23.05 ± 0.77	31.6 ± 1.6	0.88 ± 0.27	2.7 ± 0.7
1500	96	8.14 ± 0.36	8.9 ± 0.3	0.400 ± 0.017	49.2 ± 1.2	1.09 ± 0.03	22.19 ± 0.33	28.4** ± 1.2 (−3.0 s)	0.51 ± 0.10	2.4 ± 0.5
	185	7.89* ± 0.32 (+5.3)	9.3 ± 0.3	0.391 ± 0.011	49.5 ± 1.3	1.17 ± 0.03	23.68 ± 0.51	28.7** ± 0.9 (−3.6 s)	0.55 ± 0.12	2.5 ± 0.4
	367	7.80 ± 0.24	9.1 ± 0.3	0.391 ± 0.013	50.2* ± 1.2 (−2.9%)	1.16* ± 0.04 (−4.1%)	23.13 ± 0.48	28.3** ± 0.9 (−3.1 s)	0.94 ± 0.27	2.5 ± 0.5
3000	96	8.06 ± 0.26	8.7 ± 0.3	0.396 ± 0.013	49.1 ± 1.7	1.08 ± 0.04	21.96** ± 0.29 (−1.7)	27.0** ± 1.2 (−4.4 s)	0.57 ± 0.12	2.7 ± 0.8
	185	7.63 ± 0.47	8.9 ± 0.3	0.376 ± 0.019	49.3 ± 1.7	1.16 ± 0.05	23.54 ± 0.68	27.0** ± 1.8 (−5.3 s)	0.79 ± 0.27	3.2 ± 1.5
	367	7.60 ± 0.40	8.7** ± 0.3 (−5.4%)	0.380 ± 0.016	50.1* ± 1.9 (−3.0%)	1.15** ± 0.05 (−5.0%)	22.89 ± 0.43	27.4** ± 1.2 (−4.0 s)	0.85 ± 0.24	2.7 ± 0.8

From Buesen et al. (2009b)
EOS, eosinophils; HCT, haematocrit; HGB, haemoglobin; HQT, Hepato-Quick test (prothrombin time); MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; NEUTA, neutrophils; RBC, red blood cells; SD, standard deviation; * $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)

Table 61. Selected clinical chemistry findings in rats administered fluxapyroxad for 1 year (chronic toxicity group)

	Month	Group mean values ± SD									
		Males (n = 10/group)					Females (n = 10/group)				
		0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm
AST (µkat/l)	3	1.78 ± 0.22	1.84 ± 0.44	1.67 ± 0.32	1.61 ± 0.28	1.47 ± 0.18	1.65 ± 0.22	1.51 ± 0.24	1.49 ± 0.09	1.29** ± 0.46 (−22%)	1.23** ± 0.09 (−25%)

	Month	Group mean values ± SD									
		Males (*n* = 10/group)					Females (*n* = 10/group)				
		0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm
	6	1.48 ± 0.26	1.55 ± 0.32	1.48 ± 0.27	1.53 ± 0.51	1.36 ± 0.27	1.53 ± 0.24	1.41 ± 0.23	1.57 ± 0.70	1.18** ± 0.18 (−23%)	1.23** ± 0.19 (−20%)
	12	1.76 ± 1.03	2.25 ± 1.82	1.61 ± 0.44	1.72 ± 0.81	1.51 ± 0.22	2.17 ± 0.86	2.01 ± 0.57	1.80 ± 0.38	1.58 ± 0.43	1.64 ± 0.62
ALT (µkat/l)	3	0.61 ± 0.09	0.71 ± 0.14	0.65 ± 0.08	0.65 ± 0.08	0.64 ± 0.18	0.58 ± 0.09	0.57 ± 0.12	0.56 ± 0.09	0.55 ± 0.10	0.56 ± 0.09
	6	0.58 ± 0.10	0.64 ± 0.14	0.60 ± 0.08	0.61 ± 0.11	0.62 ± 0.17	0.64 ± 0.14	0.54 ± 0.15	0.56 ± 0.11	0.54 ± 0.09	0.52 ± 0.10
	12	0.64 ± 0.13	0.76 ± 0.21	0.70 ± 0.10	0.73 ± 0.19	0.82 ± 0.21	0.75 ± 0.13	0.76 ± 0.17	0.79 ± 0.14	0.67 ± 0.09	0.67 ± 0.10
ALP (µkat/l)	3	1.04 ± 0.12	1.03 ± 0.19	1.67 ± 0.32	1.61 ± 0.29	1.47 ± 0.18	0.51 ± 0.14	0.49 ± 0.13	0.44 ± 0.10	0.44 ± 0.09	0.41 ± 0.14
	6	0.89 ± 0.12	0.82 ± 0.13	0.92 ± 0.17	0.86 ± 0.15	0.86 ± 0.14	0.41 ± 0.11	0.38 ± 0.11	0.35 ± 0.15	0.35 ± 0.06	0.34 ± 0.09
	12	0.89 ± 0.10	0.75 ± 0.13	0.91 ± 0.16	0.83 ± 0.17	0.83 ± 0.14	0.38 ± 0.11	0.36 ± 0.12	0.38 ± 0.16	0.35 ± 0.06	0.33 ± 0.09
GGT (µkat/l)	3	20 ± 10	26 ± 9	28 ± 8	36* ± 18	69** ± 28	3 ± 3	4 ± 4	3 ± 4	2 ± 4	8 ± 12
	6	1 ± 3	0 ± 1	0 ± 3	22* ± 32	71** ± 41	2 ± 4	4 ± 6	1 ± 2	3 ± 5	11** ± 16
	12	7 ± 6	9 ± 7	12 ± 5	58** ± 54	134** ± 60	4 ± 5	7 ± 4	5 ± 5	7 ± 7	19 ± 22
K^+ (mmol/l)	3	4.54 ± 0.27	4.59 ± 0.31	4.51 ± 0.16	4.51 ± 0.16	4.58 ± 0.15	4.15 ± 0.18	4.13 ± 0.39	4.11 ± 0.42	4.35 ± 0.27	4.21 ± 0.22
	6	4.34 ± 0.27	4.44 ± 0.24	4.32 ± 0.21	4.43 ± 0.19	4.41 ± 0.24	4.27 ± 0.18	3.87** ± 0.38	4.01* ± 0.33	4.25 ± 0.29	4.10 ± 0.32
	12	4.56 ± 0.35	4.64 ± 0.36	4.56 ± 0.34	4.55 ± 0.37	4.48 ± 0.26	4.38 ± 0.34	4.18 ± 0.46	4.12 ± 0.38	4.22 ± 0.30	4.06 ± 0.35
Cl^- (mmol/l)	3	106.1 ± 0.7	105.4* ± 0.5 (−0.7%)	105.9 ± 1.1	104.3** ± 1.0 (−1.7%)	104.6** ± 0.9 (−2.0%)	106.6 ± 1.2	106.0 ± 1.8	105.4 ± 1.3	105.4 ± 1.1	104.7 ± 1.8
	6	101.7 ± 0.6	101.4 ± 0.9	101.5 ± 1.1	100.6* ± 1.1 (−1.1%)	100.4** ± 0.8 (−1.3%)	102.5 ± 1.2	101.9 ± 1.6	101.7 ± 1.1	101.3* ± 1.5 (−1.1%)	100.1** ± 1.8 (−2.3%)
	12	107.3 ± 0.8	106.6 ± 0.9	106.7 ± 1.3	106.5 ± 1.2	106.3 ± 1.0	105.9 ± 1.2	105.3 ± 2.7	105.3 ± 2.0	103.5** ± 1.5 (−2.2%)	103.3** ± 1.2 (−2.4%)
PO_4^{3-}, inorganic (mmol/l)	3	1.71 ± 0.13	1.67 ± 0.20	1.65 ± 0.20	1.76 ± 0.25	1.92** ± 0.13 (+12%)	1.46 ± 0.19	1.34 ± 0.23	1.28 ± 0.28	1.53 ± 0.22	1.57 ± 0.24
	6	1.56 ± 0.16	1.57 ± 0.13	1.55 ± 0.13	1.69 ± 0.13	1.86** ± 0.13 (+19%)	1.30 ± 0.25	1.18 ± 0.12	1.20 ± 0.27	1.32 ± 0.24	1.36 ± 0.18
	12	1.27 ± 0.18	1.25 ± 0.17	1.23 ± 0.16	1.30 ± 0.15	1.42 ± 0.16	1.31 ± 0.21	1.20 ± 0.28	1.14 ± 0.20	1.37 ± 0.21	1.33 ± 0.24

Table 61 (continued)

	Month	Group mean values ± SD									
		Males ($n = 10$/group)					Females ($n = 10$/group)				
		0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm
Ca^{2+} (mmol/l)	3	2.62 ± 0.07	2.65 ± 0.08	2.66 ± 0.05	2.67 ± 0.09	2.74** ± 0.08 (+4.5%)	2.63 ± 0.05	2.67 ± 0.10	2.65 ± 0.06	2.73** ± 0.04 (+3.8%)	2.82** ± 0.06 (+7.2%)
	6	2.66 ± 0.07	2.72 ± 0.07	2.71 ± 0.08	2.74* ± 0.07 (+3.0%)	2.77** ± 0.04 (+4.1%)	2.68 ± 0.06	2.65 ± 0.07	2.67 ± 0.12	2.76	2.78** ± 0.08 (+3.7%)
	12	2.64 ± 0.06	2.66 ± 0.06	2.69 ± 0.04	2.69 ± 0.05	2.71 ± 0.09	2.68 ± 0.05	2.71 ± 0.11	2.68 ± 0.06	2.79** ± 0.08 (+4.1%)	2.81** ± 0.08 (+4.9%)
Glucose (mmol/l)	3	5.90 ± 0.64	5.48 ± 0.81	5.58 ± 0.63	5.11* ± 0.76 (−13%)	4.67** ± 0.86 (−21%)	5.11 ± 0.76	5.71* ± 0.66 (+12%)	5.61* ± 0.54 (+9.8%)	5.33 ± 0.80	4.92 ± 0.28
	6	5.55 ± 0.55	5.53 ± 0.53	5.05** ± 0.37 (−9.0%)	5.11* ± 0.45 (−7.9%)	4.82* ± 0.54 (−13%)	5.13 ± 0.59	5.59 ± 1.07	5.47 ± 0.49	5.06 ± 0.50	5.02 ± 0.58
	12	5.85 ± 0.52	5.55 ± 0.45	5.66 ± 0.66	5.42 ± 0.72	4.79** ± 0.60 (−18%)	4.94 ± 0.50	5.39 ± 0.60	5.46* ± 0.29 (+11%)	5.24 ± 0.57	4.78 ± 0.38
Bilirubin, total (µmol/l)	3	2.64 ± 0.47	2.27 ± 0.39	2.12* ± 0.39 (−20%)	1.99** ± 0.42 (−25%)	1.92** ± 0.23 (−27%)	3.46 ± 0.39	3.46 ± 0.82	3.12 ± 0.54	2.55** ± 0.28 (−26%)	2.73* ± 0.65 (−21%)
	6	2.24 ± 0.39	2.12 ± 0.44	1.84 ± 0.43	1.81 ± 0.37	1.74 ± 0.46	4.02 ± 0.40	3.35** ± 0.46 (−17%)	3.00** ± 0.66 (−25%)	2.60** ± 0.62 (−65%)	2.77** ± 0.65 (31%)
	12	1.98 ± 0.37	1.71 ± 0.35	1.73 ± 0.35	1.50 ± 0.38	1.74 ± 0.39	3.43 ± 0.50	3.50 ± 1.04	3.13 ± 0.34	2.81 ± 0.95	2.20** ± 0.56 (−36%)
Protein, total (g/l)	3	63.45 ± 1.39	64.11 ± 1.67	64.55 ± 1.68	67.46** ± 2.05 (+6.3%)	69.11** ± 2.66 (+8.9%)	67.55 ± 2.56	69.15 ± 4.06	69.52 ± 3.30	71.70** ± 1.94 (+6.1%)	76.25** ± 2.40 (+13%)
	6	66.28 ± 1.78	67.17 ± 1.68	67.97 ± 1.98	69.87** ± 1.51 (+5.4%)	70.81** ± 2.09 (+6.8%)	69.93 ± 2.33	71.46 ± 3.83	72.11 ± 3.55	75.37** ± 2.35 (+7.8%)	78.79** ± 3.00 (+13%)
	12	69.47 ± 1.40	69.42 ± 1.51	69.87 ± 1.84	72.68** ± 2.09 (+4.6%)	73.80** ± 2.03 (+6.2%)	71.04 ± 2.98	72.80 ± 4.30	73.42 ± 2.02	77.42** ± 3.10 (+9.0%)	79.97** ± 2.69 (+13%)
Albumin (g/l)	3	37.45 ± 1.07	37.67 ± 0.99	37.83 ± 0.88	38.07 ± 1.19	39.18 ± 1.14	40.74 ± 1.68	41.25 ± 2.42	41.40 ± 2.28	41.30 ± 1.04	42.89 ± 1.57
	6	36.71 ± 0.94	36.95 ± 0.50	37.33 ± 0.65	37.22 ± 0.64	37.97 ± 1.25	41.04 ± 1.50	41.48 ± 1.88	41.57 ± 2.04	42.17 ± 1.25	43.11** ± 1.02 (+5.0%)
	12	37.41 ± 0.69	36.99 ± 1.03	37.49 ± 0.85	37.56 ± 0.71	38.03 ± 1.03	41.52 ± 1.43	41.73 ± 1.74	42.03 ± 0.85	42.65 ± 1.74	43.03 ± 1.41
Globulin (g/l)	3	26.01 ± 0.81	26.44 ± 0.94	26.71 ± 1.08	29.39** ± 1.46 (+13%)	29.93** ± 1.14 (+15%)	26.81 ± 1.03	27.91 ± 2.18	28.11* ± 1.35 (+4.8%)	30.40** ± 1.44 (+13%)	33.36** ± 1.40 (+24%)
	6	29.57	30.22 ±	30.64 ±	32.64** ± 1.34	32.84** ± 1.16	28.89 ±	29.98 ±	30.54 ±	33.20** ± 1.47	35.68** ± 2.40

	Month	Group mean values ± SD									
		Males (*n* = 10/group)					Females (*n* = 10/group)				
		0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm
		± 1.08	1.23	1.54	(+10%)	(+11%)	1.31	2.33	1.91	(+15%)	(+24%)
	12	32.06 ± 1.25	32.43 ± 1.25	32.39 ± 1.62	35.12** ± 1.98 (+9.5%)	35.77** ± 1.43 (+12%)	29.52 ± 1.77	31.06 ± 2.97	31.39* ± 1.82 (+6.3%)	34.77** ± 1.68 (+18%)	36.94** ± 1.68 (+25%)
Triglycerides (mmol/l)	3	0.77 ± 0.27	0.76 ± 0.23	0.69 ± 0.33	0.72 ± 0.25	0.65 ± 0.27	0.44 ± 0.09	0.47 ± 0.13	0.58 ± 0.22	0.51 ± 0.20	0.76** ± 0.35 (+73%)
	6	0.65 ± 0.36	0.69 ± 0.28	0.78 ± 0.32	0.64 ± 0.23	0.64 ± 0.27	0.56 ± 0.14	0.58 ± 0.18	0.56 ± 0.18	0.73 ± 0.18	0.82 ± 0.38
	12	1.03 ± 0.43	1.20 ± 0.44	1.13 ± 0.46	1.14 ± 0.34	0.91 ± 0.35	0.76 ± 0.34	0.75 ± 0.28	0.63 ± 0.23	0.75 ± 0.25	0.83 ± 0.30
Cholesterol (mmol/l)	3	1.70 ± 0.40	1.81 ± 0.32	2.21* ± 0.54 (+30%)	2.50** ± 0.43 (+47%)	3.05** ± 0.78 (+79%)	1.65 ± 0.35	1.80 ± 0.28	1.82 ± 0.40	2.69** ± 0.53 (+63%)	3.36** ± 0.66 (104%)
	6	1.84 ± 0.42	1.93 ± 0.39	2.37* ± 0.59 (+29%)	2.87** ± 0.59 (+56%)	3.23** ± 0.74 (+76%)	1.85 ± 0.35	1.82 ± 0.40	1.88 ± 0.47	2.66** ± 0.50 (+44%)	3.24** ± 0.53 (+75%)
	12	2.09 ± 0.44	2.18 ± 0.47	2.63* ± 0.65 (+26%)	3.22** ± 0.75 (+54%)	3.58** ± 1.06 (+71%)	1.98 ± 0.28	2.18 ± 0.36	2.03 ± 0.52	3.25** ± 0.64 (+64%)	3.69** ± 0.95 (+86%)

From Buesen et al. (2009b)
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; SD, standard deviation; * $P \le 0.05$; ** $P \le 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)

Chronic toxicity (1 year's duration) was also investigated in this study in satellite animals (10 of each sex per dose). Adverse effects on body weight were indicated by terminal body weights that were decreased more than 10% relative to untreated controls in both sexes at 3000 ppm. Increased absolute and relative liver weights were observed at and above 250 ppm in females and 1500 ppm in males (Table 62) in conjunction with centrilobular hepatocellular hypertrophy at and above 1500 ppm in males and females, pigment storage (likely lipofuscin) in males and females at and above 1500 ppm and liver discoloration at 3000 ppm in both sexes. None of these changes was considered clearly adverse.

In addition, changes in electrolyte levels (calcium, chloride and inorganic phosphate) were observed in females and/or males at 3000 ppm (phosphate in males) and at and above 1500 ppm (calcium, chloride in males and females), but were not of a magnitude considered adverse. Also, in livers of females, a decrease in PPB-stainable iron (as Fe^{3+}) was observed at and above 1500 ppm, which was not considered adverse. After 2 years of treatment, non-neoplastic changes in the livers of these animals included increases in the absolute and relative liver weights of both sexes at and above 250 ppm (Table 63) in conjunction with centrilobular hepatocellular hypertrophy, spongiosis hepatitis in males at and above 1500 ppm, increased pigment storage (likely lipofuscin) in males and females at and above 1500 ppm and dark brown liver discoloration at and above 1500 ppm (Table 64).

Table 62. Selected mean absolute and relative organ weights of rats administered fluxapyroxad for 1 year (chronic toxicity group)

	Dietary concentration (ppm)	Mean weight ± SD							
		Males (*n* = 10/group)				Females (*n* = 10/group)			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
Terminal weight (g)	0	462.4 ± 35.8	—	—	—	253.9 ± 22.6	—	—	—
	50	491.9 ± 43.4	(+6.4)	—	—	265.4 ± 31.1	(+4.5)	—	—
	250	460.1 ± 47.2	(−0.5)	—	—	252.6 ± 21.8	(−0.5)	—	—
	1500	461.1 ± 48.7	(−0.3)	—	—	232.3** ± 15.1	(−8.5)	—	—
	3000	407.3** ± 29.4	(−11.9)	—	—	225.9 ** ± 18.8	(−11.0)	—	—
Adrenal glands (mg)	0	55.7 ± 5.0	—	0.012 ± 0.001	—	66.1 ± 11.7	—	0.026 ± 0.005	—
	50	57.6 ± 4.6	(+3.4)	0.012 ± 0.001	(−3.0)	63.3 ± 9.1	(−4.2)	0.024 ± 0.003	(−8.1)
	250	52.4 ± 5.9	(−5.9)	0.011 ± 0.001	(−5.6)	64.7 ± 9.4	(−2.1)	0.026 ± 0.004	(−1.5)
	1500	59.4 ± 11.2	(+6.6)	0.013 ± 0.002	(+6.3)	62.9 ± 8.4	(−4.8)	0.027 ± 0.003	(+3.8)
	3000	60.11 ± 8.3	(+7.9)	0.015** ± 0.002	(+21.8)	60.5 ± 9.5	(−8.5)	0.027 ± 0.004	(+2.6)
Brain (g)	0	2.170 ± 0.081	—	0.472 ± 0.038	—	2.062 ± 0.104	—	0.816 ± 0.064	—
	50	2.137 ± 0.120	(−1.5)	0.436 ± 0.031	(−7.5)	2.028 ± 0.051	(−1.6)	0.774 ± 0.100	(−5.1)
	250	2.199 ± 0.052	(+1.3)	0.483 ± 0.053	(+2.4)	2.062 ± 0.075	(0.0)	0.821 ± 0.068	(+0.7)
	1500	2.126 ± 0.067	(−2.0)	0.465 ± 0.039	(−1.5)	2.054 ± 0.079	(−0.4)	0.886* ± 0.049	(+8.6)
	3000	2.146 ± 0.062	(−1.1)	0.530* ± 0.048	(+12.3)	1.987 ± 0.088	(−3.6)	0.884* ± 0.066	(+8.3)
Heart (g)	0	1.140 ± 0.074	—	0.248 ± 0.023	—	0.760 ± 0.065	—	0.300 ± 0.022	—
	50	1.165 ± 0.079	(+2.2)	0.237 ± 0.12	(−4.1)	0.781 ± 0.062	(+2.8)	0.296 ± 0.022	(−1.3)
	250	1.117 ± 0.112	(−2.0)	0.244 ± 0.020	(−1.6)	0.794 ± 0.074	(+4.5)	0.315 ± 0.022	(+5.0)
	1500	1.131 ± 0.134	(−0.8)	0.245 ± 0.014	(−0.9)	0.740 ± 0.046	(−2.6)	0.319* ± 0.013	(+6.3)
	3000	1.084 ± 0.085	(−4.9)	0.266* ± 0.007	(+7.5)	0.737 ± 0.076	(−3.0)	0.326* ± 0.018	(+8.7)
Kidneys (g)	0	2.422 ± 0.251	—	0.528 ± 0.079	—	1.724 ± 0.135	—	0.680 ± 0.037	—
	50	2.538 ± 0.237	(+4.8)	0.518 ± 0.050	(−1.9)	1.756 ± 0.166	(+1.9)	0.665 ± 0.045	(−2.3)

	Dietary concentration (ppm)	Mean weight ± SD							
		Males ($n = 10$/group)				Females ($n = 10$/group)			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
	250	2.497 ± 0.268	(+3.1)	0.545 ± 0.058	(+3.4)	1.749 ± 0.229	(+1.5)	0.692 ± 0.061	(+1.7)
	1500	2.643 ± 0.301	(+9.1)	0.575 ± 0.052	(+8.9)	1.696 ± 0.140	(−1.6)	0.732 ± 0.064	(+7.6)
	3000	2.582 ± 0.301	(+6.6)	0.635* ± 0.068	(+20.3)	1.630 ± 0.140	(−5.5)	0.724 ± 0.064	(+6.4)
Liver (g)	0	9.290 ± 0.679	—	2.017 ± 0.197	—	5.376 ± 0.480	—	2.119 ± 0.117	—
	50	9.717 ± 0.820	(+4.6)	1.981 ± 0.148	(−1.8)	5.688 ± 0.573	(+5.8)	2.152 ± 0.137	(+1.5)
	250	9.621 ± 1.112	(+3.6)	2.093 ± 0.140	(+3.7)	5.835* ± 0.473	(+8.5)	2.317** ± 0.161	(+9.3)
	1500	12.54** ± 2.12	(+35.0)	2.712** ± 0.237	(+34.4)	6.471** ± 0.540	(+20.4)	2.785** ± 0.141	(+31.4)
	3000	12.70** ± 1.39	(+36.7)	3.119** ± 0.281	(+54.7)	7.067** ± 0.540	(+31.5)	3.135** ± 0.200	(+47.9)
Spleen (g)	0	0.707 ± 0.098	—	0.153 ± 0.013	—	0.533 ± 0.078	—	0.210 ± 0.026	—
	50	0.705 ± 0.115	(−0.3)	0.143 ± 0.018	(−6.2)	0.512 ± 0.055	(−3.9)	0.196 ± 0.035	(−6.7)
	250	0.692 ± 0.093	(−2.1)	0.151 ± 0.019	(−1.1)	0.533 ± 0.067	(0.0)	0.212 ± 0.033	(+1.2)
	1500	0.707 ± 0.139	(0.0)	0.154 ± 0.026	(+0.7)	0.485 ± 0.073	(−9.0)	0.209 ± 0.031	(−0.4)
	3000	0.706 ± 0.081	(−0.1)	0.173** ± 0.017	(+13.6)	0.491 ± 0.060	(−7.9)	0.218 ± 0.024	(+3.6)
Testes (g)	0	3.819 ± 0.427	—	0.829 ± 0.102	—	—	—	—	—
	50	3.603 ± 0.491	(−5.7)	0.740 ± 0.128	(−10.7)	—	—	—	—
	250	3.937 ± 0.441	(+3.1)	0.870 ± 0.178	(+4.9)	—	—	—	—
	1500	3.657 ± 0.325	(−4.2)	0.798 ± 0.084	(−3.7)	—	—	—	—
	3000	3.886 ± 0.306	(+1.8)	0.959* ± 0.110	(+15.7)	—	—	—	—
Thyroid (mg)	0	28.2 ± 4.94	—	0.006 ± 0.001	—	18.2 ± 3.5	—	0.007 ± 0.001	—
	50	29.0 ± 5.6	(+2.8)	0.006 ± 0.001	(−3.3)	23.5** ± 2.8	(+29.1)	0.009** ± 0.002	(+26.0)
	250	29.5 ± 4.0	(+4.6)	0.006 ± 0.001	(+5.9)	20.6 ± 4.7	(+13.2)	0.008 ± 0.002	(+14.5)
	1500	31.0 ± 4.4	(+9.9)	0.007 ± 0.001	(+10.5)	19.5 ± 2.4	(+7.1)	0.008** ± 0.001	(+17.7)
	3000	31.75 ± 4.5	(+12.6)	0.008 ± 0.001**	(+25.3)	22.0 ± 4.5	(+20.9)	0.010** ± 0.002	(+36.3)

Table 62 (continued)

	Dietary concentration (ppm)	Mean weight ± SD							
		Males (*n* = 10/group)				Females (*n* = 10/group)			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
Uterus (g)	0	—	—	—	—	0.681 ± 0.118	—	0.269 ± 0.049	—
	50	—	—	—	—	0.727 ± 0.133	(+6.8)	0.276 ± 0.052	(+2.4)
	250	—	—	—	—	0.976** ± 0.279	(+43.3)	0.388** ± 0.113	(+44.2)
	1500	—	—	—	—	0.768 ± 0.255	(+12.8)	0.329 ± 0.101	(+22.1)
	3000	—	—	—	—	0.869* ± 0.225	(+27.6)	0.384** ± 0.087	(+42.6)

From Buesen et al. (2009b)
* $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)
[a] Compared with controls. Values may not calculate exactly due to rounding.
[b] Percentage of body weight.

Table 63. Selected mean absolute and relative organ weights of rats administered fluxapyroxad for 2 years (carcinogenicity group)

	Dietary concentration (ppm)	Mean values ± SD (*n*)							
		Males				Females			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
Terminal weight (g)	0	529.4 ± 61.8 (44)	—	—	—	339.9 ± 52.7 (37)	—	—	—
	50	553.6 ± 84.6 (42)	(+4.6)	—	—	322.8 ± 50.0 (40)	(−5.0)	—	—
	250	541.9 ± 65.4 (44)	(+2.4)	—	—	297.8** ± 39.7 (41)	(−12.4)	—	—
	1500	514.0 ± 60.5 (45)	(−2.9)	—	—	281.3** ± 29.1 (35)	(−17.2)	—	—
	3000	477.8** ± 54.9 (41)	(−9.7)	—	—	260.1** ± 36.3 (39)	(−23.5)	—	—
Adrenal glands (mg)	0	65.57 ± 23.9 (44)	—	0.012 ± 0.005 (44)	—	68.76 ± 11.32 (37)	—	0.020 ± 0.003 (37)	—
	50	63.02 ± 8.80 (42)	(−3.9)	0.012 ± 0.002 (42)	(−7.7)	73.82 ± 24.13 (39)	(+7.4)	0.023 ± 0.008 (39)_	(+14.1)
	250	62.82 ± 11.20 (44)	(−4.2)	0.012 ± 0.002 (44)	(−6.4)	67.46 ± 16.37 (41)	(−1.9)	0.023 ± 0.006 (41)	(+11.7)
	1500	69.69 ± 61.81 (45)	(+6.3)	0.014** ± 0.005 (45)	(+11.5)	60.86** ± 10.69 (35)	(−11.4)	0.022 ± 0.005 (35)	(+6.9)

	Dietary concentration (ppm)	Mean values ± SD (*n*)							
		Males				Females			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
	3000	83.25 ± 118.79 (40)	(+27.0)	0.018** ± 0.029 (40)	(+45.6)	62.62* ± 12.03 (39)	(−8.9)	0.024** ± 0.005 (39)	(+18.7)
Brain (g)	0	2.183 ± 0.093 (44)	—	0.417 ± 0.050 (44)	—	2.031 ± 0.084 (37)	—	0.610 ± 0.085 (37)	—
	50	2.207 ± 0.093 (42)	(+1.1)	0.407 ± 0.061 (42)	(−2.4)	2.066 ± 0.179 (40)	(+1.7)	0.655 ± 0.120 (40)	(+7.4)
	250	2.226 ± 0.101 (44)	(+2.0)	0.417 ± 0.061 (44)	(0.0)	2.040 ± 0.077 (41)	(+0.4)	0.696** ± 0.087 (41)	(+14.1)
	1500	2.210 ± 0.087 (45)	(+1.2)	0.437 ± 0.065 (45)	(+4.8)	2.013 ± 0.091 (35)	(−0.9)	0.723** ± 0.080 (35)	(+18.5)
	3000	2.213 ± 0.103 (41)	(+1.4)	0.468** ± 0.052 (41)	(+12.2)	1.999 ± 0.076 (39)	(−1.6)	0.781** ± 0.095 (39)	(+28.0)
Epididymides (g)	0	1.196 ± 0.433 (44)	—	0.230 ± 0.098 (44)	—	—	—	—	—
	50	1.116 ± 0.152 (42)	(−6.7)	0.205 ± 0.038 (42)	(−10.9)	—	—	—	—
	250	1.190 ± 0.152 (44)	(−0.5)	0.221 ± 0.027 (44)	(−3.9)	—	—	—	—
	1500	1.238 ± 0.503 (45)	(+3.5)	0.242* ± 0.102 (45)	(+5.2)	—	—	—	—
	3000	1.178 ± 0.159 (41)	(−1.5)	0.249** ± 0.038 (41)	(+8.3)	—	—	—	—
Heart (g)	0	1.345 ± 0.159 (44)	—	0.255 ± 0.025 (44)	—	1.015 ± 0.138 (37)	—	0.302 ± 0.036 (37)	—
	50	1.402 ± 0.143 (42)	(+4.2)	0.256 ± 0.028 (42)	(+0.4)	0.981 ± 0.112 (40)	(−3.3)	0.309 ± 0.043 (40)	(+2.3)
	250	1.385 ± 0.175 (44)	(+3.0)	0.256 ± 0.021 (44)	(+0.4)	0.951* ± 0.101 (41)	(−6.3)	0.322** ± 0.034 (41)	(+6.6)
	1500	1.418 ± 0.145 (45)	(+5.4)	0.280** ± 0.049 (45)	(+9.8)	0.951* ± 0.100 (35)	(−6.3)	0.341** ± 0.048 (35)	(+12.9)
	3000	1.353 ± 0.141 (41)	(+0.6)	0.285** ± 0.035 (41)	(+11.8)	0.926** ± 0.091 (39)	(−8.8)	0.360** ± 0.043 (39)	(+19.2)

Table 63 (continued)

	Dietary concentration (ppm)	Mean values ± SD (*n*)							
		Males				Females			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
Kidneys (g)	0	3.015 ± 0.326 (44)	—	0.575 ± 0.076 (44)	—	2.106 ± 0.299 (37)	—	0.626 ± 0.074 (37)	—
	50	3.156 ± 0.435 (42)	(+4.7)	0.574 ± 0.065 (42)	(−0.2)	2.193 ± 0.313 (40)	(+4.1)	0.688* ± 0.101 (40)	(+9.9)
	250	3.235* ± 0.477 (44)	(+7.3)	0.600 ± 0.077 (44)	(+4.3)	2.122 ± 0.294 (41)	(+0.8)	0.719** ± 0.100 (41)	(+14.9)
	1500	3.347** ± 0.580 (45)	(+11.0)	0.666** ± 0.206 (45)	(+15.8)	2.085 ± 0.254 (35)	(−1.0)	0.748** ± 0.117 (35)	(+19.5)
	3000	3.141 ± 0.411 (41)	(+4.2)	0.663** ± 0.097 (41)	(+15.3)	2.000 ± 0.300 (39)	(−5.0)	0.775** ± 0.122 (39)	(+23.8)
Liver (g)	0	11.28 ± 1.54 (44)	—	2.137 ± 0.223 (44)	—	7.306 ± 1.251 (37)	—	2.153 ± 0.186 (37)	—
	50	11.87 ± 2.03 (42)	(+5.2)	2.147 ± 0.240 (42)	(+0.5)	7.324 ± 1.209 (40)	(+0.2)	2.284 ± 0.299 (40)	(+6.1)
	250	12.52** ± 2.03 (44)	(+10.9)	2.307** ± 0.231 (44)	(+8.0)	7.194 ± 1.220 (41)	(−1.5)	2.418** ± 0.292 (41)	(+12.3)
	1500	14.96** ± 2.22 (45)	(+32.6)	2.953** ± 0.690 (45)	(+38.2)	8.253** ± 1.490 (35)	(+13.0)	2.937** ± 0.459 (35)	(+36.4)
	3000	16.37** ± 2.47 (41)	(+45.1)	3.448** ± 0.536 (41)	(+61.3)	8.645** ± 1.399 (39)	(+18.3)	3.329** ± 0.340 (39)	(+54.6)
Spleen (g)	0	0.966 ± 0.152 (44)	—	0.184 ± 0.030 (44)	—	0.742 ± 0.320 (37)	—	0.222 ± 0.110 (37)	—
	50	1.140 ± 0.495 (42)	(+18.0)	0.207 ± 0.087 (42)	(+12.5)	0.696 ± 0.156 (40)	(−6.2)	0.220 ± 0.062 (40)	(−0.9)
	250	1.032 ± 0.369 (44)	(+6.8)	0.190 ± 0.062 (44)	(+3.3)	0.633** ± 0.239 (41)	(−14.7)	0.217 ± 0.103 (41)	(−2.3)
	1500	1.007 ± 0.164 (45)	(+4.2)	0.199 ± 0.046 (45)	(+8.2)	0.674* ± 0.357 (35)	(−9.2)	0.243 ± 0.143 (35)	(+9.5)
	3000	0.879** ± 0.169 (41)	(−9.0)	0.186 ± 0.038 (41)	(+1.1)	0.551** ± 0.107 (39)	(−25.7)	0.214 ± 0.043 (39)	(−3.6)
Testes (g)	0	3.825 ± 0.397 (44)	—	0.730 ± 0.101 (44)	—	—	—	—	—

	Dietary concentration (ppm)	Mean values ± SD (*n*)							
		Males				Females			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
	50	4.177 ± 1.850 (42)	(+9.2)	0.771 ± 0.378 (42)	(+5.6)	—	—	—	—
	250	4.053 ± 0.945 (44)	(+6.0)	0.757 ± 0.200 (44)	(+3.7)	—	—	—	—
	1500	4.015 ± 0.893 (45)	(+5.0)	0.781 ± 0.151 (45)	(+7.0)	—	—	—	—
	3000	4.234 ± 1.288 (41)	(+10.7)	0.896** ± 0.293 (41)	(+22.7)	—	—	—	—
Uterus (g)	0	—	—	—	—	0.737 ± 0.299 (36)	—	0.222 ± 0.102 (36)	—
	50	—	—	—	—	0.972 ± 0.520 (40)	(+31.9)	0.318* ± 0.198 (40)	(+43.2)
	250	—	—	—	—	0.904 ± 0.485 (40)	(+22.7)	0.310** ± 0.170 (40)	(+39.6)
	1500	—	—	—	—	1.071 ± 0.961 (34)	(+45.3)	0.391** ± 0.364 (34)	(+76.1)
	3000	—	—	—	—	0.925 ± 0.732 (39)	(+25.5)	0.370** ± 0.347 (39)	(+66.7)

Buesen et al. (2009b)
SD, standard deviation; * $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)
[a] Compared with controls. Values may not calculate exactly due to rounding.
[b] Percentage of body weight.

Table 64. Incidence of selected gross pathological findings in rats administered fluxapyroxad for 1 year (chronic toxicity group) or 2 years (carcinogenicity group)

	Incidence of finding									
	Males					Females				
	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm
Interim sacrifice (chronic toxicity group)										
No. of animals examined	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*
Incisor										
- mandible, discoloration	0	0	2	1	5	0	0	0	0	5
Liver										
- discoloration, dark brown	0	0	0	1	7	0	0	0	1	5
Carcinogenicity group										
No. of animals examined	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*

Table 64 (continued)

	Incidence of finding									
	Males					Females				
	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm
Abdominal cavity										
- effusion	0	0	0	1	0	1	0	1	1	5
Liver										
- cyst	1	1	0	7	12	2	5	3	4	3
- discoloration, dark brown	1	0	0	2	6	0	1	0	4	16
- enlarged	0	1	0	1	7	0	0	0	1	4
- focus	32	30	40	44	41	20	17	27	25	29
- mass	0	0	3	8	16	1	2	0	6	5
- prominent acinar pattern	0	1	1	8	17	1	0	1	8	18
Bone (head)										
- discoloration, bulla tympanica	0	0	0	0	31	0	0	0	0	31
- discoloration, dorsal skull	0	0	0	0	16	0	0	0	0	21
- discoloration, frontal bone	0	0	0	0	38	0	0	0	0	34
Incisor										
- mandible, discoloration	0	0	2	32	39	0	0	1	36	39
- maxilla, discoloration	0	0	0	23	38	0	0	0	12	37
Thyroid gland										
- enlarged	1		2	2	10	1	1	0	0	0
- mass	2	0	1	3	4	1	1	1	2	1

From Buesen et al. (2009b)

In addition, the bones of animals in the carcinogenicity group were examined by gross pathology and histopathology (Tables 65 and 66). In the femora and other bones of affected animals (nasal turbinates, sterna, vertebrae), deposition of PPB stain–positive material, most probably iron, was observed at and above 250 ppm in both sexes. No changes in the bone structure were identified by light microscopy. Gross pathological evaluation identified white discoloration of skull bones (frontal bone, dorsal skull, tympanic cavity) that was diagnosed as periosteal hyperostosis (i.e. bone thickening). These effects were considered potentially adverse. Discoloration of the teeth (incisors) was also observed in 3000 ppm males and females (Table 64). This was not considered adverse.

Table 65. Incidence of non-neoplastic findings in rats administered fluxapyroxad for 1 year

	Incidence and severity[a] of finding									
	Males (n = 10/group)					Females (n = 10/group)				
	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm
Femur										
No. examined	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*
PPB stain	0	0	0	10	10	0	0	0	10	10
				[1.9]	[3.0]				[2.9]	[3.1]

	Incidence and severity[a] of finding									
	Males (*n* = 10/group)					Females (*n* = 10/group)				
	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm
Liver										
No. examined	*10*	*10*	*10*	*10*	*10*	*10*	*9*	*10*	*10*	*10*
Hypertrophy, centrilobular (zone 3)	2	0	1	10	9	0	0	5	10	10
	[1.0]		[1.0]	[1.5]	[1.7]			[1.0]	[2.3]	[3.2]
Pigment storage, diffuse	0	0	0	3	6	1	0	0	8	9
Liver lymph node hyperplasia, lymphoreticular	1	0	2	1	8	0	1	2	2	2
Thyroid										
No. examined	*10*	*10*	*10*	*10*	*10*	*10*	*9*	*10*	*10*	*10*
Hypertophy/hyperplasia, follicular	3	5	5	10	7	0	0	0	1	0
Altered colloid	6	7	8	10	9	1	1	3	8	9

From Buesen et al. (2009b)

[a] [ ] mean severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked (Grade 4) and massive/severe (Grade 5). The mean severity is the sum of the gradings divided by the incidence.

Table 66. Incidence of non-neoplastic findings in rats administered fluxapyroxad for 2 years

	Incidence and severity[a] of finding									
	Males (*n* = 50/group)					Females (*n* = 50/group)				
	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm
Femur										
No. examined	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*
PPB stain	0	0	35**	50**	50**	0	0	33**	50**	50**
			[1.0]	[2.1]	[2.9]			[1.4]	[2.9]	[3.4]
Inflammation, diffuse	3	0	0	0	7	0	0	0	0	0
Liver										
No. examined	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*
Hypertrophy, centrilobular (zone 3)	1	2	30**	43**	43**	0	0	28**	41**	44**
	[1.0]	[1.0]	[1.2]	[2.4]	[2.7]			[1.3]	[2.2]	[2.7]
Pigment storage, diffuse	2	0	0	9*	14**	18	18	26	33**	47**
Spongiosis hepatitis	0	0	1	10**	23**	0	0	0	0	0
PPB stain		0	0	0	0	42	38	30	**11**	**3**
Focus of cellular alteration	43	47	42	44	40	46	46	47	24	27
- Basophilic tigroid	37	40	35	20	9	45	45	47	9	5
- Basophilic diffuse	7	8	2	5	1	4	1	0	0	1
- Basophilic (not otherwise specified)	3	3	5	7	10*	5	2	4	3	2
- Clear cell	11	19	5	3	0	6	1	2	0	0
- Eosinophilic	33	36	39	38	35	10	10	19*	16	23**
- Amphophilic	0	0	0	0	0	0	1	0	0	0

Table 66 (continued)

	Incidence and severity[a] of finding									
	Males (n = 50/group)					Females (n = 50/group)				
	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm
Parathyroid glands										
No. examined	*50*	*50*	*50*	*48*	*50*	*45*	*46*	*49*	*46*	*47*
Hyperplasia, (multi)focal	8	7	6	6	12	1	2	3	1	3
Skull bones										
No. examined	44	—	—	—	42	39	2	2	7	40
Os frontale, hyperostosis	0	—	—	—	41	0	0	0	0	32
Os parietale, hyperostosis	0	—	—	—	0	0	0	0	0	5
Bulla tympanica, hyperostosis	0	—	—	—	4	0	0	0	0	0
Thyroid										
No. examined	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*
Hyperplasia, follicular	3	4	8	17**	14**	2	1	2	8*	4
Altered colloid	40	44	46	48*	47*	30	33	37	41**	45**

From Buesen et al. (2009b)

* $P \leq 0.05$; ** $P \leq 0.01$ (comparison of all dose groups with the control group using Fisher's exact test [one-sided] for the hypothesis of equal proportions)

[a] [ ] mean severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked (Grade 4) and massive/severe (Grade 5). The mean severity is the sum of the gradings divided by the incidence.

The thyroid as well as the femur and other bones were also affected. In the thyroid, an increased incidence of follicular cell hypertrophy/hyperplasia was observed in males at and above 1500 ppm. This was observed in conjunction with enlarged thyroid in all males at 3000 ppm. These thyroid effects were considered adverse.

Tumours considered a result of treatment were observed in the liver and thyroid only (Tables 67, 68 and 69). Hepatocellular tumours were in the form of adenomas and carcinomas in both sexes, with a significant increase in the combined incidence of these tumour types in males at and above 1500 ppm and in females at 3000 ppm. Significant increases in hepatocellular carcinomas were observed only in males and only at 3000 ppm. Adenomas were observed in both sexes at a higher incidence than carcinomas and at lower doses. Significant increases in adenomas were observed in males at and above 1500 ppm and in females at 3000 ppm. Adenoma incidence was greater than the upper limit of historical controls (Table 70) in males and females at and above 1500 ppm and so was determined to be an effect of treatment.

Thyroid tumours resulting from treatment were in the form of follicular cell adenomas and carcinomas. The combined incidence of adenomas and carcinomas was significantly increased for males only at 3000 ppm compared with concurrent controls. The incidence was within the historical control range (Table 71), but was still considered an effect of treatment. The individual incidence of thyroid follicular cell adenomas or carcinomas did not reach statistical significance for males or females. For males only, the incidence of carcinomas at 3000 ppm was not significantly different from that of concurrent controls, but was greater than the upper limit of the historical control values and so was considered treatment related. In males and females, the incidence of adenomas did not reach statistical significance at any dose compared with concurrent controls and was within the historical control range. These results were consistent with thyroid histopathology, which showed significant thyroid follicular hyperplasia in males at and above 1500 ppm. Colloid was also altered in both sexes at 1500 ppm and above.

Table 67. Incidence of primary neoplastic findings in rats administered fluxapyroxad for 1 year (chronic toxicity group)

	Incidence of finding									
	Males (*n* = 10/group)					Females (*n* = 10/group)				
	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm
Brain										
No. examined	*10*	—	—	—	*10*	*10*	—	—	—	*10*
Tumour, granular cell, benign	0	—	—	—	0	0	—	—	—	1
Glioma, mixed, malignant	0	—	—	—	1	0	—	—	—	0
Kidneys										
No. examined	*10*	—	—	—	*10*	*10*	—	—	—	*10*
Adenoma	1	—	—	—	0	0	—	—	—	0
Pituitary gland										
No. examined	*10*	—	—	—	*10*	*10*	*1*	*2*	*1*	*10*
Adenoma, pars distalis	0	—	—	—	0	0	0	1	1	0
Thyroid glands										
No. examined	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*
Adenoma, C-cell	1	0	0	1	0	0	1	0	0	0

From Buesen et al. (2009b)

Table 68. Incidence of primary neoplastic findings[a] in rats administered fluxapyroxad for up to 2 years (carcinogenicity group)

	Incidence of finding									
	Males					Females				
	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm
Liver										
No. examined	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*
Adenoma, hepatocellular	0	0	4	7**	15**	0	2	0	4	**7****
Carcinoma, hepatocellular	1	0	1	3	9**	1	1	0	0	0
Combined hepatocellular tumours	1	0	5	10**	21**[b]	1	3	0	4	**7***
Haemangiosarcoma	0	0	0	1	0	0	0	0	0	0
Mammary gland										
No. examined	*1*	*1*	*1*	—	—	*49*	*26*	*19*	*18*	*50*
Adenoma	0	0	0	—	—	1	1	0	0	0
Adenocarcinoma	0	0	0	—	—	4	5	1	1	1
Fibroadenoma	1	1	1	—	—	12	13	11	5	5
Fibroma	0	0	0	—	—	1	0	0	0	0
Histiocytoma, fibrous, malignant	0	0	0	—	—	0	0	0	1	1
Haemangioma	0	0	0	—	—	0	0	0	0	1
Pituitary gland										
No. examined	*50*	*15*	*17*	*20*	*49*	*50*	*31*	*24*	*36*	*50*
Adenoma, pars intermedia	0	0	0	1	0	0	1	0	0	0

Table 68 (continued)

	Incidence of finding									
	Males					Females				
	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm
Adenoma, pars distalis	9	10	11	15	10	30	20	15	27	12
Carcinoma, pars distalis	0	0	0	0	0	1	1	0	0	0
Schwannoma, malignant	0	0	1	0	0	0	0	0	0	0
Testes										
No. examined	*50*	*16*	*17*	*10*	*50*	—	—	—	—	—
Adenoma, Leydig cell	4	1	2	1	2	—	—	—	—	—
Mesothelioma, malignant	0	1	1	0	0	—	—	—	—	—
Thymus										
No. examined	*50*	*7*	*5*	*5*	*48*	*49*	*11*	*10*	*16*	*49*
Thymoma, benign	0	0	0	0	2	4	1	1	1	4
Thymoma, malignant	1	0	0	0	0	0	0	0	0	0
Thyroid glands										
No. examined	*50*	*50*	*50*	*50*	*50*	*50*	*49*	*50*	*48*	*50*
Adenoma, C-cell	5	6	4	2	6	13	6	6	5	8
Carcinoma, C-cell	1	0	0	0	1	0	1	0	0	0
Adenoma, follicular cell	3	2	4	8	9	0	3	1	3	2
Carcinoma, follicular cell	0	0	1	1	3	2	0	1	0	1
Combined follicular cell tumours	3	2	5	9	11*[c]	2	3	2	3	3
Uterus										
No. examined	—	—	—	—	—	*50*	*30*	*29*	*32*	*50*
Deciduoma	—	—	—	—	—	0	0	1	0	0
Adenocarcinoma, endometrial	—	—	—	—	—	8	7	8	9	11
Carcinoma, squamous cell	—	—	—	—	—	0	0	0	0	1
Sarcoma, endometrial stromal	—	—	—	—	—	2	0	1	2	1

From Buesen et al. (2009b)

* $P \leq 0.05$; ** $P \leq 0.01$ (comparison of all dose groups with the control group using Fisher's exact test [one-sided] for the hypothesis of equal proportions)

[a] This table does not list the incidences of infiltrates of systemic lymphoma and of metastases in the various organs.

[b] Three animals with adenoma and carcinoma.

[c] One animal with adenoma and carcinoma.

Table 69. Total incidence of neoplastic findings in rats administered fluxapyroxad for 2 years

	Incidence of findings									
	Males (*n* = 50/group)					Females (*n* = 50/group)				
	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm
Number of animals with:										
- neoplasms	32	32	34	33	41	46	43	36	40	40
- 1 primary neoplasm	20	22	20	16	18	17	19	20	19	20

	Incidence of findings									
	Males (n = 50/group)					Females (n = 50/group)				
	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm
- 2+ primary neoplasms	12	10	14	17	23	29	24	16	21	20
- benign neoplasms	28	26	28	28	32	42	35	30	33	29
- benign neoplasms only	24	25	25	22	19	29	27	25	24	22
- malignant neoplasms	8	7	9	11	22	17	16	11	16	18
- malignant neoplasms only	4	6	6	5	9	4	8	6	7	11
- systemic neoplasms	2	1	0	0	1	0	0	0	2	0
- metastasized neoplasms	1	1	1	1	3	3	2	2	4	7
Total number of:										
- primary neoplasms	44	42	52	61	79	95	78	63	69	71
- benign neoplasms	35	34	42	49	53	73	59	51	52	51
- malignant neoplasms	9	8	10	12	26	22	19	12	17	20
- systemic neoplasms	2	1	0	0	1	0	0	0	2	0
- metastasized neoplasms	1	1	1	1	3	4	2	2	4	7

From Buesen et al. (2009b)

Table 70. Historical control data for hepatocellular tumours in Wistar rats

	Males	Females	Strain	Time frame (month/year)
Adenoma, hepatocellular				
Fluxapyroxad study	0	0	Crl:WI(Han)	1/2007–1/2009
BASF SE	8/400 [2% (0–4%)]	3/400 [0.8% (0–6%)]	Crl:WI(Han)	8/1999–1/2008
Carcinoma, hepatocellular				
Fluxapyroxad study	1	1	Crl:WI(Han)	1/2007–1/2009
BASF SE	6/400 [1.5% (0–6%)]	7/400 [1.8% (0–6%)]	Crl:WI(Han)	8/1999–1/2008
Combined incidence of hepatocellular tumour–bearing rats				
Fluxapyroxad study	1	1	Crl:WI(Han)	1/2007–1/2009
BASF SE	14/400 [3.5% (0–8%)]	10/397 [2.5% (0–6%)]	Crl:WI(Han)	8/1999–1/2008

From Buesen et al. (2009b)

The NOAEL for chronic toxicity was 50 ppm (equal to 2.1 mg/kg bw per day for males and 2.7 mg/kg bw per day for females), based on decreased body weight in both sexes in the absence of effects on feed consumption at 250 ppm (equal to 11 mg/kg bw per day for males and 14 mg/kg bw per day for females) (Buesen et al., 2009b).

Table 71. Historical control data for thyroid follicular tumours in Wistar rats

	Males	Females	Strain	Time frame (month/year)
Adenoma, follicular cells				
Fluxapyroxad study	3	1	Crl:WI(Han)	1/2007–1/2009
BASF SE	52/400 [13% (4–28%)]	24/400 [6.0% (2–10%)]	Crl:WI(Han)	8/1999–1/2008
Carcinoma, follicular cells				
Fluxapyroxad study	0	1	Crl:WI(Han)	1/2007–1/2009
BASF SE	9/400 [2.3% (0–4%)]	4/397 [1.0% (0–4%)]	Crl:WI(Han)	8/1999–1/2008
Combined incidence of thyroid follicular cell tumour–bearing rats				
Fluxapyroxad study	3	2	Crl:WI(Han)	1/2007–1/2009
BASF SE	60/400 [15.0% (4–30%)]	28/397 [7.1% (2–12%)]	Crl:WI(Han)	8/1999–1/2008

From Buesen et al. (2009b)

2.4 Genotoxicity

A battery of GLP-compliant studies of mutagenicity with fluxapyroxad was conducted to assess its potential for inducing gene mutation, chromosomal aberration and unscheduled deoxyribonucleic acid (DNA) synthesis. The study results (summarized in Table 72) were negative. Overall, fluxapyroxad did not demonstrate any genotoxic potential.

Table 72. Genotoxicity studies with fluxapyroxad

End-point	Test object	Concentration	Purity (%)	Results	Reference
In vitro					
Reverse mutation	*Salmonella typhimurium* and *Escherichia coli*	0, 20, 100, 500, 2500 or 5000 µg/plate (+S9 and −S9)	99.4	Negative	Schulz & Landsiedel (2008a)
Mammalian cell gene mutation	Chinese hamster ovary cells (HPRT locus assay)	0, 5.0, 6.3, 10.0, 12.5, 20.0, 25.0, 50.0, 75.0 or 100.0 µg/ml (+S9 and −S9)	99.7	Negative	Schulz & Landsiedel (2007a)
Chromosomal aberration	Chinese hamster lung cells	0, 3.1, 6.3, 12.5, 25.0, 50.0, 100.0, 200.0 or 400.0 µg/ml (+S9 and −S9, 4 or 18 h)	99.7	Negative	Schulz & Landsiedel (2008b)
In vivo					
Mouse micronucleus	NMRI mice, male	0, 500, 1000 or 2000 mg/kg bw[a] Sampling time: 24 h	99.6	Negative	Schulz & Landsiedel (2006)
Unscheduled DNA synthesis	Wistar rats, male	0, 1000 or 2000 mg/kg bw Sampling times: 3 h, 14 h	99.7	Negative	Schulz & Landsiedel (2008c)

DNA, deoxyribonucleic acid; S9, 9000 × *g* rat liver supernatant
[a] Given as two doses 24 hours apart.

2.5 *Reproductive and developmental toxicity*

(a) Multigeneration studies

Rats

In a two-generation reproductive toxicity study in rats, fluxapyroxad (purity 99.7%) was administered in the diet to 25 Wistar rats of each sex per group at a target dose of 0, 10, 50 or 300 mg/kg bw per day for 10 weeks prior to mating and through lactation until weaning of the F_1 offspring. Groups of 25 male and 25 female F_1 generation offspring were then similarly treated. Overall average achieved dose levels were within the ranges 9.5–11.9, 47.661.1 and 285.4–378.5 mg/kg bw per day (Tables 73 and 74). In F_0 and F_1 parental animals, clinical signs were recorded daily, detailed clinical examinations, body weight measurement and feed consumption recording were performed approximately weekly, estrous cyclicity was monitored, the duration of gestation was recorded and the F_1 animals were examined for sexual developmental landmarks. F_1 and F_2 offspring were examined daily during the lactation period for clinical signs and mortality, and full external examinations and body weight recording were performed on days 0, 4, 7, 14 and 21 of lactation. Litters were culled to four pups of each sex on day 4 of lactation. All surviving and decedent F_0 and F_1 parental animals were subjected to necropsy and gross pathological examination. Sperm analysis (number, motility, morphology) was performed on all males, and major organs, including reproductive organs, were weighed from all surviving parental animals. The reproductive organs, pituitary and adrenals from 10 male and 10 female animals per generation from the control and high-dose groups and from all animals failing to mate and produce a litter were examined microscopically. In addition, the liver, adrenals and thyroid of all animals in both generations, the kidneys from control and high-dose F_0 generation males and the pituitary from all F_1 males in the intermediate- and high-dose groups were examined microscopically. Ovarian follicle counts were performed on females in the control and high-dose groups. Pups culled on day 4 of lactation, F_1 weanlings not selected to form the parental generation and offspring dying during lactation were subjected to gross necropsy. One male and one female weanling per litter per generation were subjected to organ weight analysis of brain, spleen, thymus and uterus. The thymus and spleen of F_1/F_2 weanlings showing significant weight decrease were examined histopathologically.

No treatment-related mortality was observed throughout the study in parental animals or offspring. Treatment-related clinical observations were restricted to a whitening of maxillar or mandibular incisors at the high dose. This effect was not considered adverse.

Table 73. Fluxapyroxad doses during the different phases of a two-generation study in male rats

Target dose (mg/kg bw per day)	Generation	Achieved dose (mg/kg bw per day)	
		Average	Minimum/maximum
10	F_0	9.5	7.2/10.2
	F_1	9.6	7.6/10.2
50	F_0	47.6	36.3/50.3
	F_1	47.7	37.4/51.8
300	F_0	285.4	211.7/306.6
	F_1	285.6	224.7/317.0

From Schneider et al. (2009a)

Table 74. Fluxapyroxad doses during the different phases of a two-generation study in female rats

Target dose (mg/kg bw per day)	Generation	Average achieved dose (mg/kg bw per day)		
		Premating period	Gestation period	Lactation period
10	F_0	9.8	10.2	11.8
	F_1	9.7	10.5	11.9
50	F_0	48.8	53.4	61.1
	F_1	47.9	53.1	61.1
300	F_0	292.9	317.8	360.2
	F_1	285.5	312.1	378.5

From Schneider et al. (2009a)

In parental animals, body weight development was impaired in F_0 and F_1 high-dose parental animals as well as in F_0 mid-dose parental females, with decreased terminal body weights of 9.2% or greater, which was considered adverse. In females, this was accompanied by a reduction in feed consumption. In males, the achieved compound intakes were in the range of 95–96% of the target dose levels, whereas in females, actual intakes were 95.2–126.2% of the target dose levels. The highest deviations in females were observed during the lactation phases. If only the premating and gestation periods are considered, the actual compound intakes were in the range of 95.2–106.8% of the target dose levels.

A number of changes were observed in the liver: changes in a number of clinical chemistry parameters (Table 75), increased absolute and relative liver weights at and above 50 mg/kg bw per day in males and females (Tables 76 and 77, respectively), discoloration, enlargement and prominent acinar pattern of the liver (Table 78) and histopathological changes. Histopathological changes consisted of increased incidence and severity of centrilobular hepatocyte hypertrophy in both sexes at and above 50 mg/kg bw per day (not considered adverse) and hepatocellular necrosis in top-dose males (considered adverse) (Table 79). Changes in clinical chemistry parameters included decreased serum ALT, AST and/or ALP (not considered adverse) and increased serum GGT activities in top-dose males and females (considered equivocal, as increases in serum levels of this enzyme can be associated with adaptive or adverse effects).

Table 75. Clinical chemistry findings of rats administered fluxapyroxad throughout two generations

	Mean value ± SD							
	Males				Females			
	0 mg/kg bw per day	10 mg/kg bw per day	50 mg/kg bw per day	300 mg/kg bw per day	0 mg/kg bw per day	10 mg/kg bw per day	50 mg/kg bw per day	300 mg/kg bw per day
F_0 generation (n = 11 or 12)								
ALT (µkat/l)	1.03 ± 0.18 (11)	1.01 ± 0.14 (11)	0.95 ± 0.08 (12)	0.85* ± 0.18 (−17%) (11)	0.98 ± 0.14 (12)	0.87* ± 0.09 (−11%) (12)	0.85* ± 0.13 (−13%) (12)	0.74** ± 0.10 (−24%) (12)
AST (µkat/l)	2.79 ± 1.17 (12)	2.51 ± 0.62 (11)	2.37 ± 0.67 (12)	2.01 ± 0.63 (11)	2.02 ± 0.61 (12)	1.74 ± 0.37 (12)	1.74 ± 0.82 (12)	1.53 ± 0.38 (12)

	Mean value ± SD							
	Males				Females			
	0 mg/kg bw per day	10 mg/kg bw per day	50 mg/kg bw per day	300 mg/kg bw per day	0 mg/kg bw per day	10 mg/kg bw per day	50 mg/kg bw per day	300 mg/kg bw per day
ALP (µkat/l)	1.82 ± 0.37 (12)	1.56 ± 0.24 (12)	1.6 ± 0.23 (12)	1.34** ± 0.29 (−26%) (12)	1.54 ± 0.60 (12)	1.57 ± 0.60 (12)	1.33 ± 0.38 (12)	1.11 ± 0.49 (12)
GGT (nkat/l)	0 ± 0 (12)	0 ± 0 (12)	0 ± 0 (12)	125** ± 87 (12)	0 ± 0 (12)	1 ± 1 (12)	1 ± 2 (12)	38** ± 21 (12)
F_1 generation (*n* = 12)								
ALT (µkat/l)	0.95 ± 0.13	1 ± 0.12	0.92 ± 0.15	0.82* ± 0.10 (−14%)	0.9 ± 0.14	0.8 ± 0.14	0.71* ± 0.17 (−21%)	0.69** ± 0.09 (−23%)
AST (µkat/l)	1.77 ± 0.29	1.67 ± 0.39	1.78 ± 0.33	1.47** ± 0.17 (−17%)	1.32 ± 0.21	1.43 ± 0.45	1.31 ± 0.28	1.07* ± 0.24 (−19%)
ALP (µkat/l)	1.7 ± 0.32	1.72 ± 0.33	1.75 ± 0.18	1.36* ± 0.28 (−20%)	1.15 ± 0.31	0.99 ± 0.31	0.93 ± 0.22	0.78** ± 0.19 (−32%)
GGT (nkat/l)	0 ± 0	0 ± 0	0 ± 0	99** ± 62	0 ± 0	0 ± 0	1 ± 3	54** ± 34

From Schneider et al. (2009a)
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; SD, standard deviation; * $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)

Table 76. Organ weights of F_0 and F_1 male parental animals

	Dose (mg/kg bw per day)	F_0 males (*n* = 24–25)				F_1 males (*n* = 25)			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
Terminal weight (g)	0	394.1 ± 27.2	—	—	—	398.4 ± 31.8	—	—	—
	10	387.2 ± 40.5	(−1.8)	—	—	403.5 ± 24.0	(+1.3)	—	—
	50	386.5 ± 25.4	(−1.9)	—	—	386.8 ± 31.2	(−2.9)	—	—
	300	356.4** ± 31.4	(−9.6)	—	—	351.2** ± 32.1	(−11.8)	—	—
Adrenal gland (mg)	0	59.12 ± 7.19	—	0.015 ± 0.002	—	66.44 ± 8.01	—	0.017 ± 0.002	—
	10	60.76 ± 7.04	(+2.8)	0.016 ± 0.002	(+4.8)	67.2 ± 9.2	(+1.1)	0.017 ± 0.002	(−0.3)
	50	61.36 ± 8.41	(+3.8)	0.016 ± 0.002	(+5.9)	67.64 ± 7.52	(+1.8)	0.018 ± 0.002	(+4.8)
	300	65.68** ± 7.85	(+11.1)	0.018** ± 0.002	(+22.8)	71.28 ± 10.06	(+7.3)	0.02** ± 0.003	(+21.6)

Table 76 (continued)

	Dose (mg/kg bw per day)	F_0 males (n = 24–25)				F_1 males (n = 25)			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
Brain (g)	0	2.069 ± 0.076	—	0.527 ± 0.032	—	2.11 ± 0.08	—	0.532 ± 0.04	—
	10	2.073 ± 0.085	(+0.2)	0.541 ± 0.062	(+2.7)	2.107 ± 0.087	(−0.1)	0.524 ± 0.033	(−1.7)
	50	2.06 ± 0.10	(−0.4)	0.535 ± 0.041	(+1.6)	2.104 ± 0.07	(−0.3)	0.547 ± 0.044	(+2.8)
	300	2.024 ± 0.098	(−2.2)	0.571** ± 0.050	(+8.4)	2.029** ± 0.085	(−3.8)	0.582** ± 0.05	(+9.2)
Cauda epididymis (g)	0	0.440 ± 0.039	—	0.112 ± 0.009	—	0.430 ± 0.044	—	0.109 ± 0.014	—
	10	0.441 ± 0.059	(+0.2)	0.115 ± 0.015	(+2.5)	0.417 ± 0.059	(−3.0)	0.104 ± 0.016	(−4.5)
	50	0.449 ± 0.042	(+2.0)	0.116 ± 0.011	(+4.2)	0.430 ± 0.045	(+0.0)	0.112 ± 0.014	(+2.9)
	300	0.430 ± 0.052	(−2.3)	0.121 ± 0.015	(+8.2)	0.398 ± 0.058	(−7.4)	0.114 ± 0.019	(+5.2)
Epididymides (g)	0	1.065 ± 0.085	—	0.271 ± 0.009	—	1.096 ± 0.092	—	0.276 ± 0.027	—
	10	1.091 ± 0.113	(+2.4)	0.284 ± 0.033	(+4.8)	1.107 ± 0.129	(+1.0)	0.275 ± 0.034	(−0.5)
	50	1.066 ± 0.079	(+0.1)	0.276 ± 0.023	(+2.2)	1.090 ± 0.082	(−0.5)	0.284 ± 0.032	(+2.5)
	300	1.062 ± 0.107	(−0.3)	0.299** ± 0.032	(+10.6)	1.014* ± 0.118	(−7.5)	0.29 ± 0.039	(+5.0)
Kidneys(g)	0	2.426 ± 0.287	—	0.617 ± 0.07	—	2.452 ± 0.238	—	0.616 ± 0.046	—
	10	2.509 ± 0.272	(+3.4)	0.650* ± 0.059	(+5.5)	2.559 ± 0.211	(+4.4)	0.636 ± 0.057	(+3.2)
	50	2.494 ± 0.223	(+2.8)	0.647 ± 0.063	(+4.9)	2.565 ± 0.233	(+4.6)	0.664** ± 0.043	(+7.8)
	300	2.456 ± 0.287	(+1.2)	0.689** ± 0.056	(+11.8)	2.512 ± 0.249	(+2.4)	0.717** ± 0.06	(+16.4)
Liver (g)	0	8.962 ± 1.006	—	2.273 ± 0.195	—	8.938 ± 0.792	—	2.244 ± 0.102	—
	10	9.264 ± 1.134	(+3.4)	2.391** ± 0.116	(+5.2)	9.674** ± 0.825	(+8.2)	2.396** ± 0.125	(+6.7)
	50	10.99** ± 0.79	(+22.7)	2.847** ± 0.157	(+25.1)	11.14** ± 2.27	(+24.6)	2.879** ± 0.158	(+28.3)
	300	14.24** ± 1.66	(+58.9)	3.995** ± 0.331	(+75.8)	13.98** ± 1.84	(+56.5)	3.981** ± 0.367	(+77.4)
Pituitary gland (mg)	0	10.92 ± 1.82	—	0.003 ± 0.0	—	10.08 ± 2.629	—	0.003 ± 0.001	—
	10	11.16 ± 2.14	(+2.2)	0.003 ± 0.0	(+4.0)	9.92 ± 2.999	(−1.6)	0.002 ± 0.001	(−2.5)
	50	10.96 ± 2.30	(+0.4)	0.003 ± 0.001	(+2.2)	10.6 ± 2.398	(+5.2)	0.003 ± 0.001	(+8.7)

	Dose (mg/kg bw per day)	F_0 males (n = 24–25)				F_1 males (n = 25)			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
	300	10.76 ± 1.99	(−1.5)	0.003 ± 0.0	(+8.3)	8.72 ± 2.407	(−13.5)	0.002 ± 0.001	(−2.2)
Prostate (g)	0	1.154 ± 0.164	—	0.294 ± 0.039	—	1.225 ± 0.155	—	0.309 ± 0.046	—
	10	1.071 ± 0.156	(−7.2)	0.278 ± 0.033	(−5.3)	1.205 ± 0.165	(−1.6)	0.299 ± 0.044	(−3.3)
	50	1.052* ± 0.17	(−8.8)	0.273 ± 0.046	(−7.0)	1.221 ± 0.226	(−0.3)	0.317 ± 0.058	(+2.3)
	300	1.008* ± 0.205	(−12.7)	0.282 ± 0.051	(−3.9)	1.101 ± 0.193	(−10.1)	0.314 ± 0.051	(+1.4)
Seminal vesicle (g)	0	1.208 ± 0.173	—	0.308 ± 0.048	—	1.186 ± 0.139	—	0.299 ± 0.037	—
	10	1.161 ± 0.181	(−3.9)	0.304 ± 0.051	(−1.6)	1.140 ± 0.186	(−3.9)	0.282 ± 0.04	(−5.6)
	50	1.206 ± 0.168	(−0.2)	0.313 ± 0.047	(+1.6)	1.126 ± 0.194	(−5.1)	0.292 ± 0.05	(−2.3)
	300	1.116 ± 0.213	(−7.6)	0.313 ± 0.054	(1.5)	1.096 ± 0.209	(−7.6)	0.313 ± 0.055	(+4.5)
Spleen (g)	0	0.606 ± 0.069	—	0.154 ± 0.017	—	0.62 ± 0.077	—	0.156 ± 0.015	—
	10	0.628 ± 0.07	(+3.6)	0.163 ± 0.014	(+5.4)	0.634 ± 0.071	(+2.3)	0.157 ± 0.015	(+0.9)
	50	0.601 ± 0.084	(−0.8)	0.156 ± 0.019	(+0.9)	0.592 ± 0.073	(−4.5)	0.153 ± 0.018	(−1.4)
	300	0.586 ± 0.088	(−3.3)	0.164 ± 0.019	(+6.7)	0.536** ± 0.077	(−13.5)	0.153 ± 0.018	(−1.9)
Testes (g)	0	3.478 ± 0.298	—	0.884 ± 0.068	—	3.649 ± 0.314	—	0.92 ± 0.09	—
	10	3.654 ± 0.449	(+5.1)	0.947* ± 0.104	(+7.2)	3.802 ± 0.439	(+4.2)	0.944 ± 0.117	(+2.7)
	50	3.496 ± 0.259	(+0.5)	0.907 ± 0.071	(+2.6)	3.724 ± 0.384	(+2.1)	0.968 ± 0.122	(+5.3)
	300	3.471 ± 0.298	(−0.2)	0.978** ± 0.088	(+10.7)	3.424* ± 0.373	(−6.2)	0.98 ± 0.122	(+6.5)
Thyroid glands (mg)	0	22.96 ± 4.005	—	0.006 ± 0.001	—	28.92 ± 5.283	—	0.007 ± 0.001	—
	10	23.08 ± 3.829	(+0.5)	0.006 ± 0.001	(+2.5)	28.8 ± 5.315	(−0.4)	0.007 ± 0.001	(+2.1)
	50	24.28 ± 3.747	(+5.7)	0.006 ± 0.001	(+7.9)	32.96* ± 6.215	(+14.0)	0.009* ± 0.002	(+17.4)
	300	25.52 ± 4.976	(+11.1)	0.007** ± 0.001	(+23.4)	33.76** ± 5.51	(+16.7)	0.01** ± 0.001	(+31.8)

From Schneider et al. (2009a)

* $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)

[a] Compared with controls. Values may not calculate exactly due to rounding of figures. The values given are based on the unrounded means.

[b] Percentage of body weight.

Table 77. Organ weights of F_0 and F_1 parental females

	Dose (mg/kg bw per day)	F_0 females (n = 23–25)				F_1 females (n = 25)			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
Terminal weight (g)	0	246.2 ± 16.5	—	—	—	239.5 ± 15.0	—	—	—
	10	241.0 ± 18.6	(−2.1)	—	—	235.4 ± 13.9	(−1.7)	—	—
	50	228.4** ± 11.8	(−7.2)	—	—	229.3** ± 13.4	(−4.3)	—	—
	300	223.6** ± 11.7	(−9.2)	—	—	213.8** ± 11.2	(−10.7)	—	—
Adrenal glands (mg)	0	80.0 ± 11.7	—	0.032 ± 0.004	—	79.72 ± 9.222	—	0.033 ± 0.004	—
	10	77.78 ± 8.88	(−2.8)	0.032 ± 0.003	(−0.5)	74.8 ± 9.979	(−6.2)	0.032 ± 0.004	(−4.8)
	50	81.56 ± 10.5	(+2.0)	0.036* ± 0.004	(+9.9)	76.88 ± 11.759	(−3.6)	0.034 ± 0.004	(+0.3)
	300	79.2 ± 7.9	(−1.0)	0.035* ± 0.003	(+9.1)	73.28 ± 9.062	(−8.1)	0.034 ± 0.004	(+2.7)
Brain (g)	0	1.959 ± 0.1	—	0.797 ± 0.045	—	1.973 ± 0.089	—	0.826 ± 0.052	—
	10	1.957 ± 0.05	(−0.1)	0.815 ± 0.053	(+2.2)	1.945 ± 0.066	(−1.4)	0.829 ± 0.049	(+0.3)
	50	1.944 ± 0.062	(−0.8)	0.853** ± 0.044	(+6.9)	1.953 ± 0.071	(−1.0)	0.854** ± 0.043	(+3.4)
	300	1.931 ± 0.072	(−1.4)	0.865** ± 0.041	(+8.5)	1.892** ± 0.074	(−4.1)	0.886** ± 0.046	(+7.3)
Kidneys (g)	0	1.816 ± 0.152	—	0.738 ± 0.043	—	1.66 ± 0.134	—	0.694 ± 0.053	—
	10	1.809 ± 0.124	(−0.4)	0.753 ± 0.051	(+2.0)	1.669 ± 0.11	(+0.5)	0.71 ± 0.048	(+2.3)
	50	1.79 ± 0.156	(−1.4)	0.784** ± 0.044	(+6.2)	1.71 ± 0.128	(+3.0)	0.748** ± 0.067	(+7.7)
	300	1.755 ± 0.164	(−3.4)	0.784** ± 0.052	(+6.2)	1.603 ± 0.108	(−3.4)	0.75** ± 0.043	(+8.1)
Liver (g)	0	7.192 ± 0.845	—	2.923 ± 0.293	—	6.02 ± 0.594	—	2.515 ± 0.205	—
	10	7.361 ± 0.956	(+2.3)	3.057 ± 0.336	(+4.6)	6.182* ± 0.371	(+2.7)	2.628** ± 0.106	(+4.5)
	50	7.926** ± 0.903	(+10.2)	3.468** ± 0.326	(+18.7)	6.579** ± 0.788	(+9.3)	2.865** ± 0.253	(+13.9)
	300	10.28** ± 1.15	(+42.9)	4.591** ± 0.396	(+57.1)	8.763** ± 0.529	(+45.6)	4.101** ± 0.192	(+63.0)
Ovaries (mg)	0	106.4 ± 17.8	—	0.043 ± 0.006	—	117.1 ± 18.7	—	0.049 ± 0.008	—
	10	103.3 ± 12.0	(−2.9)	0.043 ± 0.006	(−0.2)	117.2 ± 12.2	(+0.1)	0.05 ± 0.005	(+1.5)
	50	108.4 ± 14.9	(+1.9)	0.047** ± 0.006	(+9.9)	110.6 ± 19.2	(−5.5)	0.048 ± 0.008	(−1.7)

	Dose (mg/kg bw per day)	F_0 females (n = 23–25)				F_1 females (n = 25)			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
	300	110.7 ± 18.8	(+4.1)	0.050** ± 0.008	(+14.8)	107.9* ± 11.7	(−7.8)	0.051 ± 0.006	(+3.1)
Pituitary gland (mg)	0	14.12 ± 2.91	—	0.006 ± 0.001	—	12.6 ± 2.5	—	0.005 ± 0.001	—
	10	12.87 ± 2.46	(−8.9)	0.005 ± 0.001	(−6.3)	12.96 ± 1.46	(+2.9)	0.006 ± 0.001	(+4.6)
	50	11.68** ± 2.08	(−17.3)	0.005 ± 0.001	(−10.7)	13.48 ± 1.83	(+7.0)	0.006 ± 0.001	(+11.6)
	300	11.36** ± 1.89	(−19.5)	0.005 ± 0.001	(−11.1)	12.2 ± 1.4	(−3.2)	0.006 ± 0.001	(+8.5)
Spleen (g)	0	0.513 ± 0.08	—	0.208 ± 0.028	—	0.462 ± 0.049	—	0.193 ± 0.016	—
	10	0.486 ± 0.059	(−5.3)	0.202 ± 0.022	(−2.8)	0.478 ± 0.065	(+3.5)	0.203 ± 0.023	(+5.2)
	50	0.461* ± 0.067	(−10.1)	0.202 ± 0.026	(−2.9)	0.465 ± 0.061	(+0.6)	0.203 ± 0.029	(+5.5)
	300	0.446** ± 0.064	(−13.1)	0.199 ± 0.023	(−4.0)	0.419** ± 0.039	(−9.3)	0.196 ± 0.021	(+1.9)
Thyroid glands (mg)	0	20.12 ± 4.729	—	0.008 ± 0.002	—	18.76 ± 3.67	—	0.008 ± 0.002	—
	10	18.44 ± 6.14	(−8.4)	0.008 ± 0.003	(−5.5)	19.12 ± 3.89	(+1.9)	0.008 ± 0.002	(+3.5)
	50	19.76 ± 3.98	(−1.8)	0.009 ± 0.002	(+5.7)	20.44 ± 5.00	(+9.0)	0.009 ± 0.002	(+13.4)
	300	22.04 ± 5.00	(+9.5)	0.01** ± 0.002	(+20.5)	21.2 ± 3.6	(+13.0)	0.01** ± 0.002	(+26.2)
Uterus (g)	0	0.644 ± 0.179	—	0.263 ± 0.076	—	0.635 ± 0.199	—	0.264 ± 0.077	—
	10	0.658 ± 0.252	(+2.2)	0.272 ± 0.097	(+3.7)	0.554 ± 0.158	(−12.8)	0.235 ± 0.065	(−10.9)
	50	0.660 ± 0.209	(+2.5)	0.290 ± 0.091	(+10.3)	0.743 ± 0.259	(+17.0)	0.326* ± 0.012	(+23.5)
	300	0.575 ± 0.213	(−10.7)	0.258 ± 0.095	(−1.9)	0.593 ± 0.203	(−6.6)	0.278 ± 0.096	(+5.1)

From Schneider et al. (2009a)

* $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)

[a] Compared with controls. Values may not calculate exactly due to rounding of figures. The values given are based on the unrounded means.

[b] Percentage of body weight.

Table 78. Incidence of selected macroscopic findings in F_0 and F_1 parental rats

	Incidence of finding							
	Males				Females			
	0 mg/kg bw per day	10 mg/kg bw per day	50 mg/kg bw per day	300 mg/kg bw per day	0 mg/kg bw per day	10 mg/kg bw per day	50 mg/kg bw per day	300 mg/kg bw per day
No. of animals in group	25	25	25	25	25	25	25	25
F_0 generation								
Teeth								
- discoloration, mandibular incisor	0	0	0	25	0	0	0	25
- discoloration, maxillar incisor	0	0	0	1	0	0	0	1
Liver								
- discoloration	0	0	4	21	0	0	3	23
- enlargement	0	0	1	23	0	0	6	24
- prominent acinar pattern	0	0	0	1	0	0	0	0
F_1 generation								
Teeth								
- discoloration, mandibular incisor	0	0	0	25	0	0	0	25
- discoloration, maxillar incisor	0	0	0	25	0	0	0	25
Liver								
- discoloration	0	0	0	22	0	0	0	25
- enlargement	0	0	1	18	0	0	0	25
- prominent acinar pattern	0	0	0	0	0	0	1	0

From Schneider et al. (2009a)

Table 79. Incidence of selected histopathological lesions in F_0 and F_1 parental rats

	Incidence and severity[a] of lesion							
	Males				Females			
	0 mg/kg bw per day	10 mg/kg bw per day	50 mg/kg bw per day	300 mg/kg bw per day	0 mg/kg bw per day	10 mg/kg bw per day	50 mg/kg bw per day	300 mg/kg bw per day
No. of animals in group	25	25	25	25	25	25	25	25
F_0 generation								
Thyroid								
No. examined	25	25	25	25	25	25	25	25
Hypertrophy/hyperplasia, follicular (diffuse)	0	0	25*** [1.2]	25*** [2.0]	0	0	21*** [1.2]	25*** [2.0]
Hyperplasia, follicular (focal)	0	0	1 [2.0]	0	0	0	0	0
Secretory depletion (altered colloid)	0	0	23*** [1.2]	25*** [1.6]	0	0	18*** [1.3]	25*** [1.5]

	Incidence and severity[a] of lesion							
	Males				Females			
	0 mg/kg bw per day	10 mg/kg bw per day	50 mg/kg bw per day	300 mg/kg bw per day	0 mg/kg bw per day	10 mg/kg bw per day	50 mg/kg bw per day	300 mg/kg bw per day
Liver								
No. examined	25	25	25	25	25	25	25	25
Centrilobular hypertrophy, hepatocellular	0	10*** [1.0]	25*** [2.1]	25*** [3.2]	0	5 [1.0]	25*** [2.1]	25*** [3.3]
Necrosis, hepatocellular	0	0	0	6* [1.0]	1 [1.0]	1 [3.0]	0	0
Fatty cytoplasmic vacuolation, hepatocellular	3 [1.3]	6 [1.33]	18*** [1.1]	13*** [1.0]	2 [1.0]	4 [1.3]	8 [1.0]	5 [1.0]
Adrenals								
No. examined	25	25	25	25	25	25	25	25
Cortical hypertrophy	0	0	0	21*** [1.8]	0	1 [1.0]	1 [1.0]	17*** [1.4]
Cortical hyperplasia	0	0	0	2 [1.5]	0	0	0	0
F_1 generation								
Thyroid								
No. examined	25	25	25	25	25	25	25	25
Hypertrophy/hyperplasia, follicular (diffuse)	0	0	25*** [1.3]	25*** [2.0]	0	0	21*** [1.2]	25*** [2.0]
Secretory depletion (altered colloid)	0	0	21*** [1.3]	24*** [1.4]	0	0	17*** [1.0]	25*** [1.4]
Liver								
No. examined	25	25	25	25	25	25	25	25
Centrilobular hypertrophy, hepatocellular	0	15*** [1.0]	25*** [2.1]	25*** [3.2]	0	5 [1.0]	25*** [2.0]	25*** [3.1]
Necrosis, hepatocellular	0	0	1 [2.0]	5 [1.0]	0	1 [1.0]	1 [1.0]	0
Fatty cytoplasmic vacuolation, hepatocellular	4 [1.0]	10 [1.0]	14** [1.0]	**7** [1.0]	2 [1.0]	3 [1.0]	9* [1.0]	5 [1.4]
Adrenals								
No. examined	25	25	25	25	25	25	25	25
Cortical hypertrophy	1 [2.0]	0	0	13*** [1.5]	1 [2.0]	0	0	10*** [1.2]
Cortical hyperplasia	1 [2.0]	0	0	0	0	0	0	0

From Schneider et al. (2009a)
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.01$ (pairwise Fisher's test)
[a] [ ] mean severity grading; histopathological findings were graded very slight/minimal (Grade 1), slight (Grade 2), moderate (Grade 3), severe/marked (Grade 4) and very severe/massive/extreme (Grade 5). The mean severity is the sum of the gradings divided by the incidence.

In the thyroid, follicular hypertrophy/hyperplasia in males and females was observed at 50 mg/kg bw per day, and increased absolute and relative thyroid weights were observed at higher doses. Although thyroid effects may be secondary to liver effects, they are still considered adverse.

A limited histopathological examination of teeth indicated that the discoloration or whitening of teeth was probably due to a reduced incorporation of an iron-containing pigment into the outer enamel. Teeth discoloration did not affect the normal histomorphology of the teeth, as was also demonstrated in satellite group animals of the chronic rat and mouse studies. Thus, this treatment-related effect was not considered to be adverse.

Treatment with fluxapyroxad up to the target dose of 300 mg/kg bw per day had no effect on the estrous cycle, the number, morphology and motility of sperm or male or female fertility. Male and female fertility indices ranged between 92% and 100%, without any relationship to dose. Fluxapyroxad treatment did not affect reproductive performance, as evidenced by the absence of effects on the precoital interval or gestation length as well as gestation (91–100%) or live birth indices (97–100%). The observed numerical differences displayed no dose–response relationship and were thus not indicative of a relationship with treatment. Gross pathological and histopathological examination of the reproductive organs of apparently infertile males and females did not reveal any common cause for the lack of reproductive success, and thus the observed findings were considered to be unrelated to treatment. Finally, ovarian follicle counts did not reveal any differences between control and high-dose groups.

Survival of pups was not affected by treatment, as viability and lactation indices were in the range of 97.4–99.6% and 97.0–100%, respectively. Body weight development of mid- and high-dose F_1 pups was significantly impaired throughout lactation and was considered adverse, whereas significant effects on body weights and body weight gain of F_2 pups were observed only at the high dose from lactation day 7 onwards. Other pup parameters, such as sex ratio, clinical observations, organ weights and gross necropsy findings (Tables 80 and 81), did not reveal any treatment-related effects. The observed effects on absolute and relative brain, thymus and spleen weights were secondary to the lower terminal pup body weights. The sexual maturation of F_1 offspring was not affected by treatment. The observed numerical differences were minimal and well within the historical control range.

The NOAEL for systemic toxicity in parental males and females was 10 mg/kg bw per day, based on thyroid follicular hypertrophy/hyperplasia at 50 mg/kg bw per day.

The NOAEL for fertility and reproductive performance was 300 mg/kg bw per day, the highest dose tested. A lowest-observed-adverse-effect level (LOAEL) was not observed.

The NOAEL for offspring toxicity was 10 mg/kg bw per day, based on decreased pup body weight, body weight development and liver effects at 50 mg/kg bw per day (Schneider et al., 2009a).

Table 80. Organ weights of F_1 and F_2 pups

	Dose (mg/kg bw per day)	F_1 (*n*, males and females combined)				F_2 (*n*, males and females combined)			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
Weight on day 21 (g)	0	53.7 ± 4.1 (23)	—	—	—	49.4 ± 4.0 (23)	—	—	—
	10	53.3 ± 3.4 (22)	(−0.7)	—	—	50.7 ± 5.0 (25)	(+0.03)	—	—
	50	49.8** ± 3.0 (25)	(−7.3)	—	—	50.7 ± 3.7 (21)	(+0.03)	—	—

	Dose (mg/kg bw per day)	F_1 (*n*, males and females combined)				F_2 (*n*, males and females combined)			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
	300	43.9** ± 2.8 (24)	(−18)	—	—	43.4** ± 3.1 (24)	(−12)	—	—
Brain (g)	0	1.50 ± 0.06 (22)	—	2.79 ± 0.19 (22)	—	1.48 ± 0.05 (23)	—	2.99 ± 0.27 (23)	—
	10	1.50 ± 0.05 (22)	(+0.1)	2.80 ± 0.18 (22)	(+0.4)	1.48 ± 0.05 (25)	(+0.2)	2.95 ± 0.39 (25)	(−1.2)
	50	1.48[c] ± 0.05 (25)	(−1.6)	2.98** ± 0.19 (25)	(+6.8)	1.49 ± 0.06 (21)	(+0.3)	2.94 ± 0.19 (21)	(−1.5)
	300	1.43** ± 0.07 (24)	(−4.5)	3.3** ± 0.22 (24)	(+18.1)	1.44 ± 0.07 (24)	(−2.7)	3.34** ± 0.18 (24)	(+11.7)
Thymus (g)	0	0.254 ± 0.045 (22)	—	0.468 ± 0.058 (22)	—	0.230 ± 0.043 (23)	—	0.458 ± 0.060 (23)	—
	10	0.249 ± 0.036 (22)	(−2.0)	0.462 ± 0.058 (22)	(−1.3)	0.225 ± 0.043 (25)	(−2.2)	0.440 ± 0.059 (25)	(−3.9)
	50	0.234 ± 0.028 (25)	(−7.9)	0.472 ± 0.049 (25)	(+0.9)	0.237 ± 0.030 (21)	(+3.0)	0.466 ± 0.047 (21)	(+1.7)
	300	0.190** ± 0.020 (24)	(−25.2)	0.436 ± 0.043 (24)	(−6.8)	0.190** ± 0.030 (24)	(−17.4)	0.438 ± 0.049 (24)	(−4.4)
Spleen (g)	0	0.271 ± 0.043 (22)	—	0.501 ± 0.058 (22)	—	0.254 ± 0.044 (23)	—	0.508 ± 0.072 (23)	—
	10	0.264 ± 0.032 (22)	(−2.6)	0.491 ± 0.048 (22)	(−2.0)	0.257 ± 0.056 (25)	(+1.2)	0.503 ± 0.093 (25)	(−1.0)
	50	0.249 ± 0.039 (25)	(−8.1)	0.500 ± 0.065 (25)	(−0.2)	0.256 ± 0.052 (21)	(+0.8)	0.501 ± 0.080 (21)	(−1.4)
	300	0.188** ± 0.032 (24)	(−30.6)	0.429** ± 0.055 (22)	(−14.4)	0.192** ± 0.032 (24)	(−24.4)	0.442** ± 0.059 (24)	(−13.0)

From Schneider et al. (2009a)
* $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)
[a] Compared with controls. Values may not calculate exactly due to rounding.
[b] Percentage of body weight.
[c] Mean absolute brain weights of females were significantly decreased (−2.8%).

Table 81. Incidence of gross necropsy observations in F_1 and F_2 pups

	Incidence[a] of finding			
	0 mg/kg bw per day	10 mg/kg bw per day	50 mg/kg bw per day	300 mg/kg bw per day
F_1 pups				
No. of litters evaluated	23	22	25	25
No. of pups evaluated	222	207	229	229
- live	216	204	224	222

Table 81 (continued)

	Incidence[a] of finding			
	0 mg/kg bw per day	10 mg/kg bw per day	50 mg/kg bw per day	300 mg/kg bw per day
- stillborn	6	3	5	7
Postmortem autolysis	0[a]	3 (1)	0	0
Haemorrhagic thymus	0	0	0	1 (1)
Diaphragmatic hernia	0	0	0	1 (1)
Dilated renal pelvis	0	1 (1)	0	2 (1)
Small testes	0	0	1 (1)	0
Total pup necropsy observations	0	4 (2)	1 (1)	4 (3)
- % affected pups/litter	0 ± 0.0	2.7 ± 10.77	0.4 ± 1.82	1.4* ± 4.24
F_2 pups				
No. of litters evaluated	23	25	21	24
No. of pups evaluated	271	274	237	282
- live	267	269	237	281
- stillborn	4	5	0	1
Postmortem autolysis	0	3 (1)	0	1 (1)
Incisors sloped	0	0	1 (1)	0
Microphthalmia	0	0	0	1 (1)
Anophthalmia	0	0	0	1 (1)
Haemorrhagic thymus	0	1 (1)	0	1 (1)
Diaphragmatic hernia	0	3 (3)[b]	0	0
Empty stomach	0	0	1 (1)	0
Dilated renal pelvis	1 (1)	1 (1)	1 (1)	2 (2)
Enlarged kidney	0	1 (1)	0	0
Hydronephrosis	0	0	1 (1)	0
Hydroureter	0	0	1 (1)	0
Haemorrhagic testes	0	0	1 (1)	0
Small testes	1 (1)	0	1 (1)	0
Total pup necropsy observations	2 (2)	9 (6)	6 (6)	5 (4)
- % affected pups/litter	0.6 ± 2.14	3.3 ± 7.58	2.4* ± 3.85	2.1 ± 5.35

From Schneider et al. (2009a)

[a] Fetal incidence. Values in parentheses give litter incidence.

[b] % affected fetuses/litter statistically significant different from control.

(b) Developmental toxicity

Rats

In a developmental toxicity study, fluxapyroxad (purity 99.7%) was administered to mated female Crl:WI (Han) Wistar rats (25 per dose) via gavage from day 6 to day 19 of gestation at a dose level of 0, 25, 200 or 1000 mg/kg bw per day at 10 ml/kg bw in aqueous carboxymethylcellulose (0.5% weight per volume [w/v]). The animals were sacrificed on day 20 after mating for reproductive assessment and fetal examination. Clinical signs, body weight and feed consumption were recorded. Adult females were examined macroscopically at necropsy on day 20 after mating, and all fetuses were examined macroscopically at maternal necropsy and subsequently by detailed internal visceral examination or skeletal examination.

There were no premature deaths, and there were no clinical signs or changes in feed consumption (Table 82). A transient decrease in body weight gain was observed at and above 200 mg/kg bw per day from day 6 to day 8 (Table 82); however, overall body weight change was not affected (Table 83). Changes in haematology (Table 84) and clinical chemistry (Table 85) parameters were not considered adverse as a result of the small magnitude of the changes or lack of a dose–response relationship. The high dose of 1000 mg/kg bw per day resulted in increased (12–16%) absolute and relative thyroid weights (with correlating microscopic effects of thyroid hypertrophy and hyperplasia in 7/25 dams), which were considered adverse (Table 86). Absolute and relative liver weights were increased significantly, but slightly (9–13%), at this dose and occurred in the absence of corroborating effects on serum liver enzymes (liver histopathology was not examined). This effect was considered as most likely due to hepatocellular hypertrophy (due to extensive evidence of hepatocellular hypertrophy in conjunction with increased liver weights throughout the database for this chemical) and so was not considered adverse.

Table 82. Feed consumption and body weight development in rats administered fluxapyroxad during days 6–19 of gestation

	0 mg/kg bw per day	25 mg/kg bw per day	200 mg/kg bw per day	1000 mg/kg bw per day
Feed consumption (g/animal per day)				
Days 0–6	16.1	15.8	15.9	16.1
% change	—	*−1.9*	*−1.7*	*0.0*
Days 6–19	19.3	19.5	18.5	18.8
% change	—	*+0.5*	*−4.0*	*−3.0*
Days 0–20	18.4	18.5	17.6	18.0
% change	—	*−0.1*	*−4.3*	*−2.7*
Body weight (g)				
Day 0	166.3	166.6	162.9	164.2
% change	—	*+0.2*	*−2.0*	*−1.3*
Day 6	196.8	197.9	193.4	193.3
% change	—	*+0.6*	*−1.7*	*−1.8*
Day 8	205.2	205.5	199.8	198.8
% change	—	*+0.1*	*−2.6*	*−3.1*
Day 10	214.5	214.9	209.0	208.7
% change	—	*+0.1*	*−2.6*	*−2.7*
Day 19	273.5	273.2	266.1	264.5
% change	—	*−0.1*	*−2.7*	*−3.3*
Day 20	286.8	286.6	277.3	275.3
% change	—	*−0.1*	*−3.3*	*−4.0*
Body weight gain (g)				
Days 0–6	30.5	31.3	30.5	29.1
% change	—	*+2.6*	*0.0*	*−4.6*
Days 6–8	8.5	7.6	6.4*	5.5**
% change	—	*−10.6*	*−24.7*	*−35.3*
Days 8–10	9.3	9.4	9.2	9.9
% change	—	*+1.0*	*−1.1*	*+6.5*

Table 82 (continued)

	0 mg/kg bw per day	25 mg/kg bw per day	200 mg/kg bw per day	1000 mg/kg bw per day
Days 6–19	76.7	75.3	72.7	71.2
% change	—	*−1.8*	*−5.2*	*−7.2*
Days 0–20	120.5	120.0	114.4	111.1
% change	—	*−0.4*	*−5.1*	*−7.8*

From Buesen et al (2009c)

* $P < 0.05$; ** $P < 0.01$ (Dunnett test, two-sided)

Table 83. Mean gravid uterus weights and net body weight change of pregnant rats administered fluxapyroxad during days 6–19 of gestation

	Mean value ± SD			
	0 mg/kg bw per day ($n = 25$)	25 mg/kg bw per day ($n = 22$)	200 mg/kg bw per day ($n = 23$)	1000 mg/kg bw per day ($n = 21$)
Gravid uterus weight (g)	52.1 ± 8.7	51.2 ± 10.8	48.9 ± 10.7	47.2 ± 11.7
Carcass weight (g)	234.4 ± 12.0	234.4 ± 10.6	228.4 ± 12.1	228.1 ± 11.2
Net weight change from day 6 (g)	38.0 ± 7.9	37.5 ± 7.6	35.0 ± 7.8	34.8 ± 8.2

From Buesen et al. (2009c)

SD, standard deviation

Table 84. Selected haematology findings of pregnant rats (n = 25) administered fluxapyroxad during days 6–19 of gestation

	Group means ± SD			
	0 mg/kg bw per day	25 mg/kg bw per day	200 mg/kg bw per day	1000 mg/kg bw per day
Neutrophils (%)	33.6 ± 5.3	30.1* ± 4.8 (−10%)	30.2* ± 6.0 (−10%)	29.9** ± 4.2 (−11%)
Neutrophils (10^9/l)	1.6 ± 0.4	1.5 ± 0.4	1.4 ± 0.3	1.4 ± 0.3
Lymphocytes (%)	61.9 ± 5.4	65.9** ± 5.2 (+6.4%)	65.6* ± 6.3 (+6.0%)	66.0** ± 4.7 (+6.6%)
Lymphocytes (10^9/l)	2.9 ± 0.7	3.4 ± 0.8	3.1 ± 0.8	3.1 ± 0.6
Eosinophils (%)	1.9 ± 0.4	1.7* ± 0.3 (−11%)	1.9 ± 0.4	1.6* ± 0.4 (−16%)
Eosinophils (10^9/l)	0.09 ± 0.02	0.08 ± 0.02	0.09 ± 0.02	0.08 ± 0.02

From Buesen et al. (2009c)

SD, standard deviation; * $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)

The NOAEL for maternal toxicity was 25 mg/kg bw per day, based on a transient decrease in body weight gain from day 6 to day 8 at 200 mg/kg bw per day.

No treatment-related effects on caesarean section parameters were observed at any dose. No treatment-related external, visceral or skeletal malformations were noted. Fluxapyroxad was not teratogenic up to the highest dose tested. The NOAEL for developmental toxicity was 1000 mg/kg bw per day, the highest dose tested (Buesen et al., 2009c).

Table 85. Selected clinical chemistry findings of pregnant rats (n = 25) administered fluxapyroxad during days 6–19 of gestation

	Group means ± SD			
	0 mg/kg bw per day	25 mg/kg bw per day	200 mg/kg bw per day	1000 mg/kg bw per day
Calcium (mmol/l)	2.68 ± 0.07	2.69 ± 0.06	2.71 ± 0.07	2.75* ± 0.09 (+2.6%)
Bilirubin, total (µmol/l)	1.59 ± 0.50	1.57 ± 0.52	1.21** ± 0.63 (−24%)	0.85** ± 0.47 (−47%)
Protein, total (g/l)	62.21 ± 3.12	63.05 ± 3.08	64.18 ± 3.45	66.20** ± 2.90 (+6.4%)
Albumin (g/l)	33.98 ± 1.59	34.77 ± 1.84	35.33* ± 1.86 (+4.6%)	36.78** ± 1.39 (+8.2%)

From Buesen et al. (2009c)
SD, standard deviation; * $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)

Table 86. Mean absolute and relative liver and thyroid weights of pregnant rats (n = 25) administered fluxapyroxad during days 6–19 of gestation

	Dose (mg/kg bw per day)	Absolute weight ± SD	% change[a]	Relative weight[b] ± SD	% change[a]
Terminal weight (g)	0	234.7 ± 12.0	—	—	—
	25	231.0 ± 16.2	(−1.6)	—	—
	200	227.6* ± 11.9	(−3.0)	—	—
	1000	226.3* ± 12.1	(−3.6)	—	—
Liver (g)	0	11.12 ± 1.07	—	4.737 ± 0.388	—
	25	10.78 ± 1.76	(−3.0)	4.651 ± 0.607	(−1.8)
	200	11.16 ± 1.39	(+0.4)	4.896 ± 0.478	(+3.4)
	1000	12.15** ± 1.42	(+9.3)	5.368** ± 0.567	(+13.3)
Thyroid (mg)	0	17.16 ± 1.70	—	0.007 ± 0.001	—
	25	18.44 ± 2.47	(+7.5)	0.008* ± 0.001	(+9.3)
	200	18.24* ± 1.69	(+6.3)	0.008* ± 0.001	(+9.6)
	1000	19.24** ± 1.92	(+12.1)	0.008* ± 0.001	(+16.3)

From Buesen et al. (2009c)
SD, standard deviation; * $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)
[a] Compared with controls. Values may not calculate exactly due to rounding.
[b] Percentage of body weight.

Rabbits

In a developmental toxicity study in rabbits, fluxapyroxad (purity 99.7%) was administered to mated female Himalayan rabbits (25 per dose) via gavage, from day 6 to day 28 of gestation, at a dose level of 0, 10, 25 or 60 mg/kg bw per day at 10 ml/kg bw in aqueous carboxymethylcellulose (0.5% w/v). The animals were killed on day 29 after mating for reproductive assessment and fetal examination. Clinical signs, body weight and feed consumption were recorded. Adult females were examined macroscopically at necropsy on day 29 after mating, and all fetuses were examined macroscopically at maternal necropsy and subsequently by detailed internal visceral examination and skeletal examination.

There were no maternal mortalities. The high dose of 60 mg/kg bw per day elicited signs of maternal toxicity, as indicated by one abortion, decreased feed consumption (−21.9% during the

treatment period) (Table 87), corroborated by an increased number of animals with reduced or no defecation (Table 88), body weight loss during gestation days 9–11 and a decrease in cumulative body weight gain by 49.3% during the treatment period (Table 89). Body weights were decreased 12% by day 29, which was considered adverse. There were no effects observed at gross necropsy (Table 90). No maternal toxicity was observed at dose levels of 25 mg/kg bw per day or below.

Table 87. Body weight, body weight gain and feed consumption in rabbits administered fluxapyroxad during days 6–28 of gestation

	Mean values ± SD (*n*)			
	0 mg/kg bw per day	10 mg/kg bw per day	25 mg/kg bw per day	60 mg/kg bw per day
Body weight (g)				
Day 0	2540 ± 135.3 (23)	2539 ± 146.2 (24)	2524 ± 135.0 (25)	2519 ± 119.8 (22)
Day 6	2607 ± 169.3 (23)	2594 ± 166.3 (24)	2591 ± 153.8 (25)	2577 ± 124.4 (22)
Day 16	2749 ± 178.9 (23)	2706 ± 172.3 (24)	2697 ± 162.3 (25)	2592 ± 182.8* (−5.7%) (22)
Day 23	2803 ± 198.8 (23)	2729 ± 170.9 (24)	2754 ± 170.8 (25)	2641 ± 234.8* (−5.8%) (22)
Day 29	2911 ± 175.8 (23)	2841 ± 152.4 (23)	2856 ± 170.8 (25)	2572 ± 200.0* (−12%) (21)
Body weight gain (g)[a]				
Days 0–6	66.9	54.5	66.3	58
% change	—	*−18.5*	*−0.9*	*−13.3*
Days 6–28	280.9	216.7	233.5	142.5
% change	—	*−22.9*	*−16.9*	*−49.3***
Days 0–29	370.7	294.3	331.2	231.9
% change	—	*−20.6*	*−10.7*	*−37.4***
Feed consumption (g/animal per day)[a,b]				
Days 0–6	120.8	121.4	124.2	120.4
% change	—	*+0.4*	*+2.7*	*−0.3*
Days 6–28	101.6	95.9	98.1	79.4
% change	—	*−5.6*	*−3.5*	*−21.9*
Days 0–29	104.9	100.8	102.9	88.2
% change	—	*−3.9*	*−1.9*	*−16.0*

From Buesen et al. (2009d)

SD, standard deviation; * $P \leq 0.05$; ** $P \leq 0.01$ (Dunnett-test, two-sided)

[a] Standard deviations not provided in study report. % change is compared with controls.

[b] Mean of means.

Table 88. Clinical observations in rabbits administered fluxapyroxad during days 6–28 of gestation

Dose (mg/kg bw per day)	Animal no.	Gestation day	Observation
0 (control)	None		
10	38	26	Found dead
	46	27–29	Reduced defecation
25	62	29	Reduced defecation
60	79	9	Gavage error/death
	81	18–24 25–26	Reduced defecation No defecation
	82	19–20 21–29 29	Reduced defecation No defecation Abortion/sacrificed
	83	20–24	Reduced defecation
	85	17–22	Reduced defecation
	86	17–22	Reduced defecation
	93	14, 16–19	Reduced defecation
	94	14–25	Reduced defecation
	98	16–17	Reduced defecation

From Buesen et al. (2009d)

Table 89. Mean gravid uterus weights and net body weight change of pregnant rabbits administered fluxapyroxad during days 6–28 of gestation

	Mean value ± SD			
	0 mg/kg bw per day (n = 23)	10 mg/kg bw per day (n = 23)	25 mg/kg bw per day (n = 25)	60 mg/kg bw per day (n = 21)
Gravid uterus weight (g)	359.6 ± 75.98	318.0 ± 86.95	339.0 ± 83.25	271.3** ± 113.97 (−25%)
Carcass weight (g)	2551.5 ± 164.43	2522.8 ± 167.84	2516.5 ± 146.32	2480.6 ± 178.35
Net weight change from day 6 (g)	−55.7 ± 69.90	−76.4 ± 108.93	−74.1 ± 76.65	−99.1 ± 109.44

From Buesen et al. (2009d)
SD, standard deviation; ** $P \leq 0.01$ (Dunnett-test, two-sided)

Table 90. Gross necropsy findings in rabbits administered fluxapyroxad during days 6–28 of gestation

Dose (mg/kg bw per day)	Animal no.	Observation
0 (control)	3	Empty stomach
	9, NP	Blind ending uterine horn (bilateral)
	16	Absent lung lobe (lobus inferior medialis)
	22	Absent lung lobe (lobus inferior medialis)
10	37	Lungs with petechiae
	38[a]	Empty stomach, no faeces in small intestines
	43	Blind ending uterine horn (unilateral)
	46	Empty stomach

Table 90 (continued)

Dose (mg/kg bw per day)	Animal no.	Observation
25	55	Caecum with yellow dots in caecal wall
	62	Umbilical hernia (protrusion of a small part of intestine) Stomach filled to distension with clear fluid Watery faeces
	67, 70, 71, 73	Absent lung lobe (lobus inferior medialis)
60	79[b]	Thoracic cavity filled with bloody fluid
	81, NP	Stomach filled to distension with feed; no faeces in rectum
	86	No faeces in rectum
	87	Empty stomach
	92	Absent lung lobe (lobus inferior medialis)
	95	Blind ending uterine horn (unilateral); absent gallbladder
	99	Kidney with white congregation in the renal pelvis (unilateral); empty stomach
	100	Empty stomach

From Buesen et al. (2009d)
NP, not pregnant
[a] Died intercurrently.
[b] Died after gavage error.

There was an increase in total post-implantation loss and early resorptions at 60 mg/kg bw per day. Although two dams had single implantations that were early resorptions, resulting in 100% resorptions, the incidence was still increased if these dams were removed. No other treatment-related effects on caesarean section parameters were observed at any dose. No treatment-related external, visceral or skeletal malformations were noted. The only treatment-related variation was a statistically significant increase in incidence of mean per cent affected fetuses with paw hyperflexion at the high dose level (10.3%) (Table 91). This variation is frequently observed in control fetuses (0–6.7% affected fetuses per study, with a mean of 2.1%) and may reverse, as tendons stretch postnatally as limbs grow and are being used. Although this variation occurred in the presence of substantial maternal toxicity and so may be secondary to maternal toxicity, it was an effect of treatment and so is considered potentially adverse. The incidence exceeds the historical control range on both a fetal (range 0–5.3%, mean 2.1%) and litter (range 0–40%, mean 10.6%) basis.

Table 91. Incidence of external malformations and variations

	Incidence of finding			
	0 mg/kg bw per day	10 mg/kg bw per day	25 mg/kg bw per day	60 mg/kg bw per day
No. of litters evaluated	23	23	24	19
No. of fetuses evaluated	163	138	155	118
No. live	163	138	155	118
No. dead	0	0	0	0
Total external malformations				
- fetal incidence [*N* (%)]	0 (0.0)	0 (0.0)	1 (0.6)	1 (0.8)
- litter incidence	0 (0.0)	0 (0.0)	1 (4.2)	1 (5.3)
- affected fetuses/litter (mean ± SD) (%)	0.0 ± 0.00	0.0 ± 0.00	2.1 ± 10.21	0.7 ± 2.87

	Incidence of finding			
	0 mg/kg bw per day	10 mg/kg bw per day	25 mg/kg bw per day	60 mg/kg bw per day
Individual external malformations				
Absent claw				
- fetal incidence [*N* (%)]	0 (0.0)	0 (0.0)	1 (0.6)	0 (0.0)
- litter incidence [*N* (%)]	0 (0.0)	0 (0.0)	1 (4.2)	0 (0.0)
- affected fetuses/litter (mean ± SD) (%)	0.0 ± 0.00	0.0 ± 0.00	2.1 ± 10.21	0.0 ± 0.00
Meningocele				
- fetal incidence [*N* (%)]	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.8)
- litter incidence [*N* (%)]	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.3)
- affected fetuses/litter (mean ± SD) (%)	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.7 ± 2.87
Spina bifida				
- fetal incidence [*N* (%)]	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.8)
- litter incidence [*N* (%)]	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.3)
- affected fetuses/litter (mean ± SD) (%)	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.7 ± 2.87
Total external variations				
- fetal incidence [*N* (%)]	6 (3.7)	5 (3.6)	4 (2.6)	14 (12)
- litter incidence [*N* (%)]	4 (17)	4 (17)	3 (13)	8 (42)
- affected fetuses/litter (mean ± SD) (%)	3.1 ± 7.40	3.5 ± 8.51	2.4 ± 6.95	10.9* ± 15.43
Individual external variations				
Head: Subcutaneous oedema				
- fetal incidence [*N* (%)]	0 (0.0)	1 (0.7)	0 (0.0)	1 (0.8)
- litter incidence [*N* (%)]	0 (0.0)	1 (4.3)	0 (0.0)	1 (5.3)
- affected fetuses/litter (mean ± SD) (%)	0.0 ± 0.00	0.5 ± 2.61	0.0 ± 0.00	0.7 ± 2.87
Paw hyperflexion				
- fetal incidence [*N* (%)]	6 (3.7)	4 (2.9)	4 (2.6)	13 (11)
- litter incidence [*N* (%)]	4 (17)	3 (13)	3 (13)	8 (42)
- affected fetuses/litter (mean ± SD) (%)	3.1 ± 7.40	2.9 ± 8.30	2.4 ± 6.95	10.3* ± 14.47

From Buesen et al. (2009d)
SD, standard deviation; * $P \leq 0.05$ (Wilcoxon test, one-sided)

The NOAEL for maternal toxicity was 25 mg/kg bw per day, based on decreased body weight at 60 mg/kg bw per day. The NOAEL for developmental toxicity was 25 mg/kg bw per day, based on increased early resorptions, decreased fetal body weights and an increased incidence of paw hyperflexion, which were considered potentially adverse, at 60 mg/kg bw per day (Buesen et al., 2009d).

2.6 Special studies

(a) Effects on hepatic drug-metabolizing enzyme induction and cell proliferation

The objective of the studies by Buesen et al. (2009e, 2010a) was to investigate the effects of fluxapyroxad on hepatic drug-metabolizing enzyme induction and cell proliferation in rats following dietary administration for up to 14 days, to support the development of a proposed mechanism of action for possible neoplastic effects in the liver. A detailed discussion on the mode of action can be found in Appendix 1.

Groups of 10 Wistar rats of each sex per dose were treated orally, by admixture in the diet, for 14 days with fluxapyroxad (purity 99.2%) at a concentration of 0, 250, 1500 or 3000 ppm (equal to 0,

16, 96 and 192 mg/kg bw per day for males and 0, 19, 126 and 234 mg/kg bw per day for females, respectively). An additional 10 animals of each sex per dose were treated with 0 or 3000 ppm for 14 days followed by a 28-day recovery period. Body weight and feed consumption was determined weekly. Thyroid hormone measurements were conducted prior to termination. All animals were killed and subjected to necropsy. The hepatic microsomal fraction was subjected to analysis of protein content, cytochrome P450 (CYP) content and ethoxycoumarin *O*-deethylase (ECOD) and pentoxyresorufin *O*-dealkylase (PROD) activities.

There were no deaths or treatment-related clinical signs at any dose level. There were no effects of treatment on body weight gain or feed consumption in males. There were minor effects on feed consumption and body weight development of high-dose females (Table 92). Treatment with fluxapyroxad resulted in a significant increase in absolute and relative liver weights in all dose groups (Table 93). This increase was histopathologically accompanied by a dose-dependent increase in the incidence and severity of centrilobular hypertrophy, which is considered an adaptive (not adverse) response. In mid- and high-dose males, a slight, not strictly dose-dependent, increase in thyroid weights was observed (Table 93). This was accompanied by a dose-dependent increase in the incidence and severity of follicular cell hypertrophy/hyperplasia (Table 94). All observed liver and thyroid findings described above were reversible, as was determined in satellite groups administered either control diet or fluxapyroxad at a dietary concentration of 3000 ppm for 2 weeks followed by a 4-week recovery period.

Table 92. Mean body weight and body weight gain of rats administered fluxapyroxad for 14 days

	Mean value ± SD							
	Males (n = 10 or 20)				Females (n = 10 or 20)			
	0 ppm	250 ppm	1500 ppm	3000 ppm	0 ppm	250 ppm	1500 ppm	3000 ppm
Main group animals								
Body weight (g)								
- day 0	306.4 ± 8.7	305.6 ± 7.3	304.8 ± 10.4	305.0 ± 9.3	206.1 ± 7.5	200.6 ± 9.0	202.1 ± 8.3	204.6 ± 7.5
- day 14	345.2 ± 15.2	344.3 ± 10.7	348.4 ± 14.3	341.4 ± 14.8	229.4 ± 8.7	222.3 ± 8.8	225.0 ± 6.7	223.0 ± 9.0
% change[a]	—	*−0.3*	*+0.9*	*−1.1*	—	*−3.1*	*−1.9*	*−2.8*
Overall body weight gain (g)	38.8	38.7	43.6	36.4	23.4	21.7	22.9	18.5**
% change[a]	—	*−0.1*	*+12.4*	*−6.2*	—	*−7.2*	*−2.1*	*−21.1*
Recovery animals (n = 10)								
Body weight (g)								
- day 0[b,c]	307.2	—	—	305.2	206.9	—	—	204.0
- day 41 males or day 39 females	397.0 ± 21.3	—	—	390.9 ± 19.8	245.9 ± 8.8	—	—	239.6 ± 11.9
% change[a]	—	—	—	*−1.5*	—	—	—	*−2.6*
Overall body weight gain (g)	89.7 ± 13.2	—	—	85.7 ± 12.9	39.0 ± 5.8	—	—	35.5 ± 7.4
% change[a]	—	—	—	*−4.5*	—	—	—	*−8.9*

From Buesen et al. (2009e)

SD, standard deviation; ** $P \leq 0.010$ (Dunnett's test, two-sided)

[a] Compared with controls. Values may not calculate exactly due to rounding. Mean values given in the table are based on unrounded means.

[b] Mean body weight of recovery group animals only.

[c] Standard deviations not reported.

Table 93. Mean absolute and relative liver and thyroid weights of rats administered fluxapyroxad for 14 days

Sex	Dietary concentration (ppm)	Mean value ± SD							
		Males ($n = 10$)				Females ($n = 10$)			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
Main group animals									
Terminal weight (g)	0	321.9 ± 10.4	—	—	—	208.5 ± 8.4	—	—	—
	250	319.7 ± 10.7	(−0.7)	—	—	202.6 ± 9.6	(−2.8)	—	—
	1500	323.6 ± 12.3	(+0.5)	—	—	206.0 ± 7.5	(−1.2)	—	—
	3000	317.2 ± 13.1	(−1.4)	—	—	200.7 ± 9.1	(−3.7)	—	—
Liver (g)	0	7.728 ± 0.532	—	2.399 ± 0.102	—	5.258 ± 0.389	—	2.521 ± 0.144	—
	250	8.240* ± 0.667	(+6.6)	2.577* ± 0.178	(+7.4)	5.430 ± 0.346	(+3.3)	2.687* ± 0.244	(+6.6)
	1500	10.44** ± 0.71	(+35.1)	3.225** ± 0.146	(+34.4)	6.322** ± 0.346	(+20.2)	3.071** ± 0.177	(+21.8)
	3000	11.73** ± 1.17	(+51.7)	3.693** ± 0.291	(+53.9)	7.552** ± 0.925	(+43.6)	3.772** ± 0.535	(+49.6)
Thyroid glands (g)	0	0.021 ± 0.002	—	0.006 ± 0.001	—	0.017 ± 0.003	—	0.008 ± 0.001	—
	250	0.022 ± 0.003	(+7.7)	0.007 ± 0.001	(+8.3)	0.017 ± 0.002	(−2.4)	0.008 ± 0.001	(+0.7)
	1500	0.025** ± 0.003	(+19.7)	0.008* ± 0.001*	(+18.9)	0.018 ± 0.004	(+8.3)	0.009 ± 0.002	(+9.6)
	3000	0.023 ± 0.004	(+12.0)	0.007* ± 0.001	(+13.3)	0.019 ± 0.003	(+9.5)	0.009 ± 0.002	(+14.2)
Recovery group animals									
Terminal weight (g)	0	377.9 ± 19.2	—	—	—	228.9 ± 8.2	—	—	—
	3000	370.5 ± 19.2	(−2.0)	—	—	225.5 ± 12.5	(−1.5)	—	—
Liver (g)	0	8.105 ± 0.708	—	2.143 ± 0.114	—	5.828 ± 0.301	—	2.548 ± 0.140	—
	3000	9.075** ± 0.546	(+12.0)	2.452** ± 0.137	(+14.4)	5.775 ± 0.482	(−0.9)	2.561 ± 0.167	(+0.5)
Thyroid glands (g)	0	0.022 ± 0.003	—	0.006 ± 0.001	—	0.016 ± 0.001	—	0.007 ± 0.001	—
	3000	0.024 ± 0.004	(+8.2)	0.006 ± 0.001	(+10.4)	0.020* ± 0.004	(+21.0)	0.009** ± 0.001	(+22.1)

From Buesen et al. (2009e)

SD, standard deviation; * $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)

[a] Compared with controls. Values may not calculate exactly due to rounding.

[b] Percentage of body weight.

Table 94. Incidence of selected macropathological and histopathological lesions in rats administered fluxapyroxad for 14 days

	Incidence and severity[a] of lesion							
	Males				Females			
	0 ppm	250 ppm	1500 ppm	3000 ppm	0 ppm	250 ppm	1500 ppm	3000 ppm
No. of animals in group	10	10	10	10	10	10	10	10
Macropathology/main group								
Liver								
No. examined	10	10	10	10	10	10	10	10
Enlargement	0	0	0	10	0	0	0	10
				[3.9]				[3.2]
Histopathology/main group								
Liver								
No. examined	10	10	10	10	10	10	10	10
Centrilobular hypertrophy	1	6	9	10	0	2	10	10
	[1.0]	[1.2]	[1.7]	[2.4]		[1.0]	[1.9]	[2.6]
Thyroid gland								
No. examined	10	10	10	10	10	10	10	10
Follicular hypertrophy/hyperplasia	0	2	4	5	0	0	0	4
		[1.5]	[1.8]	[2.2]				[1.0]
Altered colloid	0	1	1	2	0	0	0	0
		[1.0]	[2.0]	[1.0]				
Histopathology/recovery group								
Thyroid gland								
No. examined	10	—		10	10	—	—	10
Follicular hypertrophy/hyperplasia	2	—	—	3	0	—	—	0
	[1.0]			[1.0]				
Altered colloid	1	—	—	1	0	—	—	0
	[1.0]			[1.0]				

From Buesen et al. (2009e)

[a] [ ] mean severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked/severe (Grade 4) and massive/extreme (Grade 5). The mean severity is the sum of the gradings divided by the incidence.

Analysis of blood thyroid hormone levels did not reveal any relevant alterations of triiodothyronine (T_3) or thyroxine (T_4) levels. However, TSH levels were significantly increased in high-dose males. This increase was reversible within the recovery period (Table 95).

Fluxapyroxad treatment resulted in a dose-dependent increase in total hepatic CYP content by a factor of approximately 2 as well as an increase in various phase I (Table 96) and phase II enzyme activities (Table 97). Specifically, a minimal, up to 3-fold, induction of EROD activities and a massive induction of PROD (approximately 20-fold) and benzyloxyresorufin *O*-dealkylase (BROD) activities (approximately 10-fold) were observed in high-dose males. The induction of phase I enzymes in females was comparable to that in males; however, as a result of considerably lower initial enzyme activities, the relative increases in females were higher (3-, 125- and 127-fold for EROD, PROD and BROD, respectively), although the absolute activities were lower than in males. This induction pattern resembles that of phenobarbital (PB).

Table 95. Mean hormone levels in rats administered fluxapyroxad for 14 days

	Mean hormone level ± SD (nmol/l)			
	0 ppm	250 ppm	1500 ppm	3000 ppm
Main group males	**(*n* = 20)**	**(*n* = 10)**	**(*n* = 10)**	**(*n* = 20)**
T_3	1.15 ± 0.21	1.16 ± 0.25	1.33 ± 0.18	1.27 ± 0.27
T_4	59.58 ± 6.35	55.51 ± 11.69	62.81 ± 7.56	55.05 ± 8.44
TSH	5.34 ± 2.06	5.61 ± 2.86	6.51 ± 1.94	8.03** ± 2.57 (+50%)
Main group females	**(*n* = 20)**	**(*n* = 10)**	**(*n* = 10)**	**(*n* = 20)**
T_3	1.30 ± 0.24	1.00** ± 0.24 (−30%)	1.15 ± 0.41	1.14 ± 0.30
T_4	58.16 ± 13.53	49.20 ± 15.40	53.00 ± 12.63	51.05 ± 14.50
TSH	4.17 ± 1.57	4.36 ± 1.05	4.36 ± 1.45	4.34 ± 1.24
Recovery group males	**(*n* = 10)**	**—**	**—**	**(*n* = 10)**
T_3	1.34 ± 0.10	—	—	1.46 ± 0.23
T_4	57.99 ± 9.09	—	—	61.45 ± 10.42
TSH	6.88 ± 1.87	—	—	7.08 ± 2.22
Recovery group females	**(*n* = 10)**	**—**	**—**	**(*n* = 10)**
T_3	1.56 ± 0.26	—	—	1.41 ± 0.33
T_4	41.87 ± 12.30	—	—	40.10 ± 12.42
TSH	5.15 ± 1.34	—	—	5.20 ± 1.27

From Buesen et al. (2009e)

SD, standard deviation; T_3, triiodothyronine; T_4, thyroxine; TSH, thyroid stimulating hormone; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)

Table 96. Mean cytochrome P450 content and selected phase I enzyme activities

	Dietary concentration (ppm)	Males (*n* = 10)		Females (*n* = 10)	
		Mean ± SD[a]	Relative value (%)	Mean ± SD[a]	Relative value (%)
CYP					
Main group	0	0.730 ± 0.150	—	0.548 ± 0.108	—
	250	0.923 ± 0.155	126**	0.726 ± 0.185	132*
	1500	1.453 ± 0.191	199**	1.036 ± 0.274	189**
	3000	1.538 ± 0.232	211**	1.002 ± 0.151	183**
Recovery group	0	0.755 ± 0.118	—	0.567 ± 0.144	—
	3000	0.766 ± 0.134	101	0.609 ± 0.139	107
EROD					
Main group	0	23.84 ± 4.28	—	5.57 ± 1.34	—
	250	43.67 ± 9.13	183**	9.26 ± 2.10	166**
	1500	73.93 ± 10.64	310**	15.27 ± 3.39	274**
	3000	74.35 ± 13.71	312**	15.31 ± 3.04	275**
Recovery group	0	24.88 ± 4.66	—	4.81 ± 1.42	—
	3000	23.06 ± 7.53	93	9.14 ± 2.65	190**

Table 96 (continued)

	Dietary concentration (ppm)	Males ($n = 10$)		Females ($n = 10$)	
		Mean ± SD[a]	Relative value (%)	Mean ± SD[a]	Relative value (%)
PROD					
Main group	0	19.11 ± 3.84	—	1.75 ± 0.84	—
	250	75.14 ± 24.15	393**	41.54 ± 13.82	2375**
	1500	335.30 ± 75.51	1755**	185.55 ± 64.59	10 608**
	3000	390.64 ± 87.67	2044**	219.34 ± 87.30	12 540**
Recovery group	0	19.83 ± 3.82	—	1.50 ± 0.81	—
	3000	24.04 ± 7.41	121	3.96 ± 4.39	265
BROD					
Main group	0	59.27 ± 11.40	—	2.74 ± 1.87	—
	250	193.33 ± 48.14	326**	89.80 ± 28.24	3277**
	1500	533.11 ± 44.81	899**	318.71 ± 71.06	11 632**
	3000	589.21 ± 81.23	994**	347.67 ± 81.06	12 688**
Recovery group	0	62.26 ± 11.12	—	1.93 ± 1.85	—
	3000	68.12 ± 19.90	109	9.06 ± 10.19	470**

From Buesen et al. (2009e)

CYP, cytochrome P450; BROD, benzyloxyresorufin *O*-dealkylase; EROD, ethoxyresorufin *O*-dealkylase; PROD, pentoxyresorufin *O*-dealkylase; SD, standard deviation; ** $P < 0.01$ (Wilcoxon test with Bonferoni-Holm adjustment)

[a] Units are nmol/mg protein for CYP and pmol/min per milligram protein for the enzymes.

Table 97. Mean enzyme activity of selected phase II enzymes in rats administered fluxapyroxad for 14 days

	Dietary concentration (ppm)	Males ($n = 10$)		Females ($n = 10$)	
		Mean ± SD (FU/min per milligram protein)	Relative value (%)	Mean ± SD (FU/min per milligram protein)	Relative value (%)
MUF-GT					
Main group	0	81 712 ± 21 124	—	90 984 ± 26 929	—
	250	132 625 ± 28 724	162**	141 080 ± 34 342	155**
	1500	279 713 ± 51 864	342**	278 259 ± 28 122	306**
	3000	383 257 ± 63 053	469**	336 786 ± 56 328	370**
Recovery group	0	74 632 ± 17 183	—	92 903 ± 25 951	—
	3000	110 383 ± 25 314	148**	100 022 ± 41 254	108
HOBI-GT					
Main group	0	68 024 ± 11 584	—	80 165 ± 12 056	—
	250	129 122 ± 22 503	190**	147 150 ± 29 453	184**
	1500	259 497 ± 34 079	381**	235 841 ± 39 388	294**
	3000	317 377 ± 64 785	467**	251 857 ± 33 998	314**

	Dietary concentration (ppm)	Males ($n = 10$)		Females ($n = 10$)	
		Mean ± SD (FU/min per milligram protein)	Relative value (%)	Mean ± SD (FU/min per milligram protein)	Relative value (%)
Recovery group	0	67 311 ± 14 313	—	87 584 ± 18 426	—
	3000	77 831 ± 11 946	116	85 407 ± 19 717	98
		Mean increase of T_4-UDP-GT (% control)		Mean increase of T_4-UDP-GT (% control)	
T_4-UDP-GT					
Main group	0	100.00		100.00	
	250	110.45 ± 29		181.12 ± 134	
	1500	152.12** ± 57		237.74** ± 176	
	3000	158.47** ± 59		268.41** ± 183	
Recovery group	0	100.00 ± —		100.00 ± —	
	3000	106.61 ± 31		115.76 ± 52	

From Buesen et al. (2009e)
FU, fluorescence units; HOBI-GT, 4-hydroxybiphenyl-glucuronosyl transferase; MUF-GT, 4-methylumbelliferone-glucuronosyl transferase; SD, standard deviation; T_4-UDP-GT, thyroxine uridine diphosphate-glucuronosyl transferase; ** $P < 0.01$ (Wilcoxon test with Bonferoni-Holm adjustment)

After a 4-week recovery period, enzyme activities returned to control levels in high-dose males. In females, EROD and BROD activities were still slightly, but statistically significantly, increased by a factor or 1.9 and 4.7, respectively. However, the decrease of BROD activities from a 127-fold to 4.7-fold increase relative to controls is especially considerable.

With regard to phase II enzyme induction, a statistically significant increase in the T_4-specific glucuronosyl transferase activity was observed in males and females at and above 1500 ppm. This increase is in line with the weight and histological changes observed in the thyroids of rats as well as the results of a perchlorate discharge assay. The activities of the two other glucuronosyl transferases, 4-methylumbelliferone-glucuronosyl transferase (MUF-GT) and 4-hydroxybiphenyl-glucuronosyl transferase (HOBI-GT), were significantly increased in all treated groups. Within a 4-week recovery period, all measured phase II enzyme activities returned to control levels (Buesen et al., 2009e).

Groups of 10 Wistar rats of each sex per dose were treated orally, by admixture in the diet, for 14 days with fluxapyroxad (purity 99.2%) at a concentration of 0 or 50 ppm (equal to doses of 0 and 3.0 mg/kg bw per day for males and 0 and 3.8 mg/kg bw per day for females, respectively). Body weight and feed consumption were determined weekly. Thyroid hormone measurements were conducted prior to termination. All animals were killed and subjected to necropsy. The hepatic microsomal fraction was subjected to analysis of protein content, CYP content and ECOD and PROD activities.

There were no effects on clinical signs, feed consumption, body weights or absolute or relative liver or thyroid weights (Table 98). No overall liver enzyme induction was observed, as total CYP content as well as EROD, PROD, MUF-GT and T_4-specific glucuronosyl transferase activities in both sexes were unaltered at 50 ppm (Tables 99 and 100). However, some liver enzymes were weakly induced, as indicated by an increase in BROD (both sexes) and HOBI-GT (males) activities. As no pathological changes were noted (including organ weights), the results indicate that fluxapyroxad at a dose level of 50 ppm (equal to 3.0 mg/kg bw per day in males and 3.8 mg/kg bw per day in females) does not interfere with normal homeostatic conditions (Buesen et al., 2010a).

Table 98. Mean absolute and relative liver and thyroid weights of rats administered fluxapyroxad for 14 days

	Dietary concentration (ppm)	Mean value ± SD							
		Males				Females			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
Main group animals									
Terminal weight (g)	0	338.1 ± 15.2	—	—	—	205.2 ± 11.9	—	—	—
	50	338.0 ± 16.3	(0.0)	—	—	202.5 ± 5.7	(−1.3)	—	—
Liver (g)	0	8.89 ± 0.73	—	2.627 ± 0.136	—	5.462 ± 0.879	—	2.668 ± 0.460	—
	50	10.76 ± 2.49	(+21.1)	3.176 ± 0.676	(+20.9)	4.94 ± 0.27	(−9.6)	2.440 ± 0.124	(−8.6)
Thyroid (mg)	0	21.6 ± 3.5	—	0.006 ± 0.001	—	19.2 ± 3.7	—	0.009 ± 0.002	—
	50	23.1 ± 2.6	(+6.9)	0.007 ± 0.001	(+7.4)	18.2 ± 3.5	(−5.2)	0.009 ± 0.002	(−3.8)

From Buesen et al. (2010a)
[a] Compared with controls. Values may not calculate exactly due to rounding.
[b] Percentage of body weight.

Table 99. Cytochrome P450 content and selected phase I enzyme activities in rats administered fluxapyroxad for 14 days

Dietary concentration (ppm)	Males		Females	
	Mean ± SD[a]	Relative value (%)	Mean ± SD[a]	Relative value (%)
CYP				
0	0.494 ± 0.079	—	0.393 ± 0.086	—
50	0.495 ± 0.096	100	0.393 ± 0.084	100
EROD				
0	16.74 ± 7.28	—	5.38 ± 3.07	—
50	19.97 ± 8.36	119	6.16 ± 3.00	115
PROD				
0	10.71 ± 8.22	—	0.76 ± 0.38	—
50	9.25 ± 7.21	86	1.33 ± 0.92	175
BROD				
0	35.31 ± 7.51	—	1.20 ± 0.23	—
50	47.39 ± 9.95	134**	5.06 ± 2.31	421**

From Buesen et al. (2010a)
CYP, cytochrome P450; BROD, benzyloxyresorufin *O*-dealkylase; EROD, ethoxyresorufin *O*-dealkylase; PROD, pentoxyresorufin *O*-dealkylase; SD, standard deviation; ** $P < 0.01$ (Wilcoxon test)
[a] Units are nmol/mg protein for CYP and pmol/min per milligram protein for the enzymes.

Table 100. Enzyme activity of selected phase II enzymes in rats administered fluxapyroxad for 14 days

Dietary concentration (ppm)	Males		Females	
	Mean ± SD (FU/min per milligram protein)	Relative value (%)	Mean ± SD (FU/min per milligram protein)	Relative value (%)
MUF-GT				
0	83 548 ± 25 913	—	88 341 ± 20 664	—
50	85 592 ± 18 083	102	82 010 ± 19 073	95
HOBI-GT				
0	42 697 ± 11 199	—	55 757 ± 14 097	—
50	52 544 ± 9 754	123*	62 448 ± 9 698	112
	Mean increase of T_4-GT (% control)		Mean increase of T_4-GT (% control)	
T_4-UDP-GT (AU/min per milligram protein)				
0	100		100	
50	105 ± 16		129 ± 61	

From Buesen et al. (2010a)
AU, area units; FU, fluorescence units; HOBI-GT, 4-hydroxybiphenyl-glucuronosyl transferase; MUF-GT, 4-methylumbelliferone-glucuronosyl transferase; SD, standard deviation; T_4-UDP-GT, thyroxine uridine diphosphate-glucuronosyl transferase; * $P < 0.05$ (Wilcoxon test)

The objective of a study by Buesen et al. (2009f) was to investigate the effects of fluxapyroxad on thyroid hormone levels in rats following dietary administration for 4 weeks, to support the development of a proposed mechanism of action for possible effects on the liver and thyroid gland.

Groups of 10 male and 10 female Wistar rats were treated orally, by admixture in the diet, for 28 days with fluxapyroxad (purity 99.2%) at a concentration of 0, 50, 250, 1500 or 3000 ppm (equal to doses of 0, 3.5, 19, 105 and 214 mg/kg bw per day for males and 0, 4.4, 20, 117 and 237 mg/kg bw per day for females, respectively). Blood was sampled at study days −3, 3, 7, 14, 21 and 28 for the determination of T_3, T_4 and TSH. At the end of the study, thyroid and liver weights were determined.

Treatment with fluxapyroxad did not result in clinical observations of systemic toxicity or in effects on feed consumption or body weight development, except for a temporary body weight loss at study day 6 and an overall decrease in cumulative body weight gain by approximately 20% in females at the top dose level of 3000 ppm (Table 101).

In a first analytical run, TSH, T_3 and T_4 levels were determined in blood samples collected at study days −3, 3, 7, 14, 21 and 28 in control and top-dose animals (Table 102). Changes in hormone levels were observed for TSH in both sexes and for T_4 in males, whereas no statistically or biologically significant alterations of T_3 or T_4 levels were noted in females. The data indicated that 3000 ppm caused slight elevations in TSH in males only by day 14, which remained to day 28, with no observable effects on T_3. T_4 was decreased at day 28 only.

In a second analytical run, TSH and T_4 (males only) hormone levels were determined in all dose groups (Table 103). Significant changes in thyroid hormone levels were restricted to increases in TSH levels in top-dose males and females and a decrease in T_4 levels in top-dose males. No statistically or biologically significant alterations of TSH and T_4 levels were noted at dose levels of 1500 ppm or below. The data indicated that 3000 ppm caused slight elevations in TSH in males only by day 14, which remained until day 28. This was accompanied by slight decreases in T_4 levels, which were also depressed by day 14 and remained depressed until day 28. There were no observable effects on T_3 level.

Table 101. Mean body weight and body weight gain of rats administered fluxapyroxad for 28 days

	Mean value ± SD									
	Males (n = 10)					Females (n = 10)				
	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm
Body weight (g)										
- day 0	301.5 ± 10.5	301.7 ± 11.8	298.9 ± 10.9	300.1 ± 7.3	297.0 ± 10.5	200.4 ± 7.5	200.9 ± 7.1	201.6 ± 7.2	201.0 ± 5.9	201.5 ± 9.8
- day 27	354.3 ± 22.7	349.6 ± 16.7	358.1 ± 15.2	354.4 ± 17.2	349.4 ± 19.2	223.2 ± 9.0	220.7 ± 6.6	222.2 ± 9.8	219.1 ± 9.7	219.7 ±11.1
% change[a]	—	*−1.3*	*+1.1*	*0.0*	*−1.4*	—	*−1.1*	*−0.4*	*−1.8*	*−1.5*
Overall body weight gain[b] (g)	52.9	47.9	59.3	54.2	52.5	22.7	19.9	20.6	18.1	18.3
% change[a]	—	*−9.3*	*+12.2*	*+2.6*	*−0.7*	—	*−12.7*	*−9.5*	*−20.3*	*−19.7*

From Buesen et al. (2009f)
[a] Compared with control.
[b] Standard deviations not reported.

Table 102. Mean hormone levels in rats administered fluxapyroxad for 28 days (initial analysis)

Sampling day	Mean hormone levels ± SD					
	T_3 (nmol/l)		T_4 (nmol/l)		TSH (µg/ml)	
	0	3000	0	3000	0	3000
Males (n = 10)						
−3	0.84 ± 0.11	0.91 ± 0.12	55.76 ± 7.36	58.04 ± 12.04	3.91 ± 1.71	3.27 ± 1.95
3	0.87 ± 0.18	0.92 ± 0.18	62.77 ± 6.67	66.47 ± 12.47	3.75 ± 1.20	4.27 ± 1.57
7	0.71 ± 0.17	0.85 ± 0.14	51.15 ± 12.16	48.06 ± 9.55	4.00 ± 1.72	4.40 ± 1.77
14	0.83 ± 0.16	0.80 ± 0.10	60.30 ± 9.99	55.28 ± 8.36	3.52 ± 1.26	7.39** ± 4.56 (+110%)
21	0.89 ± 0.24	0.89 ± 0.11	64.67 ± 10.01	60.30 ± 7.78	4.12 ± 1.20	7.03 ± 4.96 (+70%)
28	0.74 ± 0.12	0.71 ± 0.06	75.82 ± 12.11	62.69* ± 8.74 (−17.3%)	3.57 ± 1.31	5.82 ± 3.96 (+63%)
Females (n = 10)						
−4	1.03 ± 0.23	1.01 ± 0.20	48.00 ± 13.75	40.24 ± 10.93	2.08 ± 0.42	2.37 ± 0.88
3	1.11 ± 0.22	0.87 ± 0.31	51.75 ± 12.25	47.51 ± 19.64	2.19 ± 0.85	2.49 ± 1.28
7	0.88 ± 0.25	0.78 ± 0.18	47.24 ± 12.41	41.77 ± 13.54	2.23 ± 0.52	2.11 ± 0.72
14	0.92 ± 0.15	1.08 ± 0.27	51.41 ± 10.22	48.09 ± 13.55	2.48 ± 0.52	2.82 ± 0.88
21	0.86 ± 0.15	0.92 ± 0.26	56.40 ± 21.59	47.11 ± 10.63	2.74 ± 0.72	3.60* ± 1.28 (+31%)
28	1.03 ± 0.31	1.04 ± 0.27	58.94 ± 16.00	53.22 ± 21.48	2.64 ± 0.56	3.03 ± 0.76 (+15%)

From Buesen et al. (2009f)
SD, standard deviation; T_3, triiodothyronine; T_4, thyroxine; TSH, thyroid stimulating hormone; * $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)

Table 103. Mean hormone levels in rats administered fluxapyroxad for 28 days (second analysis)

Sampling day	Mean hormone levels ± SD				
	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm
T_4 (nmol/l) in males (*n* = 10)					
7	51.90 ± 8.61	59.87 ± 8.37	55.52 ± 9.56	58.04 ± 8.17	48.90 ± 8.13
14	65.22 ± 9.73	73.89 ± 11.44	68.28 ± 14.14	67.86 ± 13.60	52.33** ± 6.43 (−19%)
21	62.87 ± 7.82	71.94 ± 11.54	72.27 ± 11.75	66.50 ± 11.03	54.64 ± 8.63 (−13%)
28	70.50 ± 8.94	74.79 ± 13.06	69.32 ± 6.84	71.17 ± 14.80	60.44* ± 7.33 (−14%)
TSH (µg/ml) in males (*n* = 10)					
−3	5.38 ± 1.65	ND	ND	ND	5.22 ± 2.46
3	6.08 ± 1.58	ND	ND	ND	6.29 ± 2.16
7	5.88 ± 1.60	5.44 ± 1.22	4.59 ± 1.34	6.06 ± 1.75	6.56 ± 2.39
14	5.47 ± 0.93	5.62 ± 2.68	4.95 ± 1.47	6.35 ± 3.08	9.26* ± 5.70 (+69%)
21	5.39 ± 1.49	5.65 ± 1.92	5.81 ± 2.55	8.00 ± 3.61	9.09 ± 6.55 (+68%)
28	4.40 ± 1.78	6.16 ± 3.24	5.39 ± 1.28	7.38 ± 3.32	6.88 ± 4.40 (+56%)
TSH (µg/ml) in females (*n* = 10)					
−3	3.96 ± 0.75	ND	ND	ND	3.86 ± 1.56
3	3.83 ± 0.71	ND	ND	ND	4.17 ± 1.17
7	4.11 ± 0.62	3.41 ± 1.13	4.07 ± 0.71	4.23 ± 0.78	3.84 ± 1.56
14	4.28 ± 0.77	3.56* ± 0.67	4.02 ± 0.97	4.92 ± 1.05	4.83 ± 1.25
21	4.49 ± 0.68	3.45** ± 0.74	4.11 ± 0.72	4.67 ± 0.74	5.14 ± 1.64
28	4.25 ± 0.90	3.44 ± 1.08	3.92 ± 0.53	4.43 ± 1.30	4.46 ± 1.01

From Buesen et al. (2009f)

ND, not determined; SD, standard deviation; T_4, thyroxine; TSH, thyroid stimulating hormone; * $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)

The hormone level changes were accompanied by significantly increased absolute and relative thyroid weights (considered adverse) in top-dose males as well as increased absolute and/or relative liver weights (Table 104) and liver enlargement (Table 105) in both sexes at and above 1500 ppm and increased relative liver weights in males at 250 ppm (not considered adverse).

The data from this study support the hypothesis that increased conjugation and elimination of circulating T_4 lead to compensatory release of TSH from the pituitary, secondary to fluxapyroxad liver enzyme induction.

In this study, the NOAEL for the induction of thyroid hormone alterations was 1500 ppm (equal to 105 mg/kg bw per day in males and 117 mg/kg bw per day in females) (Buesen et al., 2009f).

Table 104. Mean absolute and relative liver and thyroid weights of rats administered fluxapyroxad for 28 days

	Dietary concentration (ppm)	Mean value ± SD							
		Males (n = 10)				Females (n = 10)			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
Terminal weight (g)	0	330.5 ± 23.1	—	—	—	204.6 ± 7.4	—	—	—
	50	325.9 ± 15.9	(−1.4)	—	—	202.8 ± 8.0	(−0.9)	—	—
	250	331.9 ± 14.9	(+0.4)	—	—	205.6 ± 7.7	(+0.5)	—	—
	1500	328.6 ± 16.1	(−0.6)	—	—	202.0 ± 7.9	(−1.3)	—	—
	3000	322.7 ± 18.5	(−2.4)	—	—	203.1 ± 9.7	(−0.7)	—	—
Liver (g)	0	7.445 ± 1.12	—	2.245 ± 0.212	—	4.865 ± 0.239	—	2.378 ± 0.064	—
	50	7.484 ± 0.533	(+0.5)	2.297 ± 0.123	(+2.3)	4.859 ± 0.327	(−0.1)	2.398 ± 0.169	(+0.8)
	250	8.13 ± 0.66	(+9.2)	2.447* ± 0.115	(+9.0)	5.085 ± 0.427	(+4.5)	2.473 ± 0.183	(+4.0)
	1500	9.877** ± 1.211	(+32.7)	2.998** ± 0.221	(+33.5)	5.935** ± 0.431	(+22.0)	2.937** ± 0.153	(+23.5)
	3000	11.15** ± 0.99	(+49.8)	3.451** ± 0.155	(+53.7)	7.253** ± 0.811	(+49.1)	3.564** ± 0.273	(+49.9)
Thyroid glands (mg)	0	20.1 ± 3.0	—	0.006 ± 0.001	—	18.4 ± 2.9	—	0.009 ± 0.001	—
	50	19.7 ± 3.2	(−2.0)	0.006 ± 0.001	(−0.6)	16.7 ± 2.7	(−9.2)	0.008 ± 0.001	(−8.4)
	250	22.2 ± 3.8	(+10.4)	0.007 ± 0.001	(+10.1)	18.1 ± 1.9	(−1.6)	0.009 ± 0.001	(−2.0)
	1500	23.0 ± 3.5	(+14.4)	0.007 ± 0.001	(+15.5)	20.0 ± 4.8	(+8.7)	0.010 ± 0.002	(+10.1)
	3000	24.9** ± 4.2	(+23.9)	0.008** ± 0.001	(+27.4)	19.3 ± 2.4	(+4.9)	0.010 ± 0.001	(+5.6)

From Buesen et al. (2009f)

SD, standard deviation; * $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)

[a] Compared with controls. Values may not calculate exactly due to rounding of figures. The per cent values in this table are based on unrounded means.

[b] Percentage of body weight.

The objective of a study by Buesen et al. (2009g) was to investigate the effects of fluxapyoxrad on thyroid function in rats following dietary administration for 2 weeks, to support the development of a proposed mechanism of action for possible effects on the liver and thyroid gland. In order to determine whether the stimulating effects of fluxapyroxad on thyroid hormone synthesis were direct (due to interfering with thyroid hormone biosynthesis) or indirect (by increasing metabolism of thyroid hormone), a perchlorate discharge assay was performed. In this assay, animals are administered the test chemical or positive control substances long enough to induce thyroid hormone perturbations, followed by administration of ^{125}I for several hours. This is sufficient time for a measurable fraction of the ^{125}I to be transported into the thyroid and incorporated into thyroglobulin

Table 105. Incidence of macropathological lesions in rats administered fluxapyroxad for 28 days

	Incidence of finding									
	Males					Females				
	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm
No. of animals in group	10	10	10	10	10	10	10	10	10	10
Diaphragm										
Hernia diaphragmatica	1	0	0	0	0	0	0	0	0	0
Liver										
Enlargement	0	0	0	1	9	0	0	0	1	10
Torsion	0	0	2	0	1	0	0	0	0	1

From Buesen et al. (2009f)

(organification). Subsequent administration of perchlorate blocks further uptake of ^{125}I from the blood in individuals with a normally functioning thyroid hormone biosynthetic pathway. In individuals with a faulty biosynthetic pathway that results in incomplete organification of ^{125}I, perchlorate administration causes diffusion of ^{125}I out of the thyroid (in addition to inhibiting ^{125}I uptake). Positive controls that allow determination of direct or indirect effects of a xenobiotic on thyroid hormone synthesis are propylthiouracil (PTU) and PB. PTU directly inhibits thyroglobulin iodination and the coupling of iodotyrosines within the gland. Consequently, iodide will not be incorporated into the thyroid, reducing the synthesis of hormones. Upon perchlorate administration, ^{125}I will be discharged from the thyroid. PB, a hepatic enzyme inducer, increases the thyroid hormone clearance, resulting in indirect stimulation of thyroid hormone synthesis via a compensatory stimulation of the thyroid gland by increased circulating levels of TSH. The compensatory increase in hormone synthesis is associated with an increased uptake of iodide by the thyroid gland. As this iodide is incorporated into thyroglobulin, administration of perchlorate does not result in a significant discharge of ^{125}I.

Two groups of six young male Wistar rats were treated orally, by admixture in the diet, for 14 days with fluxapyroxad (purity 99.2%) at a concentration of 0 or 3000 ppm (equal to doses of 0 or 283 mg/kg bw per day for males and 0 and 247 mg/kg bw per day for females, respectively) and then sacrificed and subjected to necropsy. Additional groups were treated orally with 2000 ppm PTU (equal to 231 and 192 mg/kg bw per day for males and females, respectively) or 1000 ppm PB (equal to 87 and 97 mg/kg bw per day for males and females, respectively) for 14 days. At the end of the 14-day period, rats were injected with ^{125}I for 6 hours, followed by injection of half of the animals in each dose group with saline or perchlorate. Animals were sacrificed 2.5 minutes later, and radioactivity was quantified in the thyroid and blood. Body weights, body weight gain and thyroid weights were recorded. Gross pathology and histopathology were not performed.

Treatment of fluxapyroxad at a dietary dose of 3000 ppm did not affect body weight or body weight gain compared with the controls (Table 106). Statistically significant decreases in body weight and body weight gain were observed in PTU-treated animals. The significantly higher body weights and body weight gains of PB-treated females (sodium chloride treatment) were not considered to be biologically relevant.

Table 106. Mean body weight and body weight gain of rats administered fluxapyroxad, PTU or PB for 14 days

	Mean value ± SD			
	Males ($n = 6$)		Females ($n = 6$)	
	Day 0	Day 14	Day 0	Day 14
NaCl treatment groups				
Group 00, control				
Group body weight (g)	178.2 ± 6.1	266.7 ± 5.9	153.0 ± 5.1	177.2 ± 4.7
Body weight gain (g)	—	88.5 ± 2.6	—	24.2 ± 4.1
Group 10, fluxapyroxad				
Group body weight (g)	173.3 ± 8.7	252.3 ± 25.8	151.0 ± 4.7	183.6 ± 5.5
Body weight gain (g)	—	79.0 ± 19.8	—	32.6 ± 6.7
Group 20, PTU				
Group body weight (g)	177.2 ± 8.3	202.4** ± 11.4 (−24.1%)	151.2 ± 7.1	159.5** ± 4.9 (−10.0%)
Body weight gain (g)	—	25.3** ± 7.8 (−71.5%)	—	8.3** ± 7.3 (−65.6%)
Group 30, PB				
Group body weight (g)	179.7 ± 6.0	275.5 ± 6.7	154.9 ± 7.3	195.7** ± 7.9 (+10.4%)
Body weight gain (g)	—	95.8 ± 6.2	—	40.8** ± 6.9 (+68.5%)
$KClO_4$ treatment groups				
Group 01, control				
Group body weight (g)	175.3 ± 7.5	276.0 ± 11.7	149.9 ± 8.0	185.1 ± 8.1
Body weight gain (g)	—	100.7 ± 6.0	—	35.2 ± 5.3
Group 11, fluxapyroxad				
Group body weight (g)	178.1 ± 6.6	266.3 ± 10.7	153.1 ± 9.2	180.3 ± 10.2
Body weight gain (g)	—	88.2* ± 6.3 (−12.4%)	—	27.3 ± 8.3
Group 21, PTU				
Group body weight (g)	175.9 ± 8.2	217.0** ± 13.2 (−21.4%)	154.0 ± 8.8	160.8** ± 4.6 (−13.1)
Body weight gain (g)	—	41.1** ± 8.1 (−59.2%)	—	6.8** ± 6.5 (−80.6)
Group 31, PB				
Group body weight (g)	176.3 ± 7.5	274.1 ± 14.3	151.5 ± 7.6	188.6 ± 8.9
Body weight gain (g)	—	97.8 ± 7.4	—	37.1 ± 7.6

From Buesen et al. (2009g)

SD, standard deviation; * $P \leq 0.05$; ** $P \leq 0.01$ (Dunnett's test, two-sided)

The PTU and PB positive controls performed as expected. Administration of PTU in the diet for 14 days caused significant (10–13%) decreases in the body weights and body weight gains of males and females (Table 106) and a significant (315–400%) increase in the thyroid weights of both sexes (Tables 107, 108, 109 and 110). The amount of ^{125}I in the thyroid was significantly lower than in control animals fed a normal diet, and treatment with perchlorate further reduced ^{125}I counts in the thyroid, as anticipated. Administration of PB in the diet for 14 days caused a significant (46%) increase in the thyroid weights of males and a 38% increase in the thyroid weights of females that did

not reach statistical significance. The amount of ^{125}I in the thyroid was significantly greater in PB-treated males than in control animals fed a normal diet, whereas no significant difference was observed for females, and the amount of ^{125}I in the thyroid was unchanged by perchlorate.

Table 107. Thyroid weights and ^{125}I counts in male rats administered sodium chloride prior to sacrifice

		Group 00 ($n = 6$) Control + NaCl	Group 10 ($n = 6$) Fluxapyroxad + NaCl	Group 20 ($n = 6$) PTU + NaCl	Group 30 ($n = 6$) PB + NaCl
Thyroid weight (g)	Mean	0.013	0.014	0.054**	0.019**
	SD	0.001	0.001	0.017	0.002
	% change	—	+8	+315	+46
Blood count (cpm)	Mean	3624	3710	5256**	3598
	SD	323	384	818	426
	% change	—	+2	+45	−1
Thyroid count (cpm)	Mean	112 521	160 461*	96 089	236 419**
	SD	21 489	31 124	8777	36 102
	% change	—	+43	−15	+110
Specific blood count (cpm/g)	Mean	3530	3666	5119**	3513
	% change	—	+4	+45	0
Specific thyroid count (cpm/g)	Mean	8 386 671	11 104 584*	1 791 599**	12 519 983**
	% change	—	+32	−79	+49
Ratio of specific thyroid/blood counts	Mean	2404	3033	372**	3674*
	SD	536	468	65	974
	% change	—	+26	−85	+53

From Buesen et al. (2009g)
cpm, counts per minute; SD, standard deviation; * $P < 0.05$; ** $P < 0.01$ (Wilcoxon test, two-sided)

Table 108. Thyroid weights and ^{125}I counts in female rats administered sodium chloride prior to sacrifice

		Group 00 ($n = 6$) Control + NaCl	Group 10 ($n = 6$) Fluxapyroxad + NaCl	Group 20 ($n = 6$) PTU + NaCl	Group 30 ($n = 6$) PB + NaCl
Thyroid weight (g)	Mean	0.008	0.012	0.040**	0.011
	SD	0.003	0.002	0.008	0.001
	% change	—	+50	+400	+38
Blood count (cpm)	Mean	5206	4980	6478*	6118
	SD	713	365	888	1600
	% change	—	−4	+24	+18
Thyroid count (cpm)	Mean	121 521	178 109	106 805	138 229
	SD	81 352	35 214	20 715	28 054
	% change	—	+47	−12	+14
Specific blood count (cpm/g)	Mean	5097	4896	6317*	5845
	% change	—	−4	+24	+15
Specific thyroid count (cpm/g)	Mean	14 611 796	15 487 743	2 641 512**	12 938 716
	% change	—	+6	−82	−11

Table 108 (continued)

		Group 00 ($n = 6$) Control + NaCl	Group 10 ($n = 6$) Fluxapyroxad + NaCl	Group 20 ($n = 6$) PTU + NaCl	Group 30 ($n = 6$) PB + NaCl
Ratio of specific thyroid/blood counts	Mean	2929	3204	423**	2316
	SD	1487	611	27	621
	% change	—	+9	−86	−21

From Buesen et al. (2009g)
cpm, counts per minute; SD, standard deviation; * $P < 0.05$; ** $P < 0.01$ (Wilcoxon test, two-sided)

Table 109. Thyroid weights and ^{125}I counts in male rats administered potassium perchlorate prior to sacrifice

		Group 01 ($n = 6$) Control + $KClO_4$	Group 11 ($n = 6$) Fluxapyroxad + $KClO_4$	Group 21 ($n = 6$) PTU + $KClO_4$	Group 31 ($n = 6$) PB + $KClO_4$
Thyroid weight (g)	Mean	0.013	0.015	0.050**	0.017*
	SD	0.002	0.004	0.009	0.002
	% change	—	+15	+285	+31
Blood count (cpm)	Mean	3950	4014	5850**	4215
	SD	313	563	785	774
	% change	—	+2	+48	+7
Thyroid count (cpm)	Mean	96 736	191 522**	32 382**	195 589**
	SD	34 054	46 444	3833	47 277
	% change	—	+98	−67	+102
Specific blood count (cpm/g)	Mean	3891	3944	5765**	4180
	% change	—	1	48	7
Specific thyroid count (cpm/g)	Mean	7 557 512	12 382 902**	653 073**	11 561 918**
	% change	—	+64	−91	+53
Ratio of specific thyroid/blood counts	Mean	1937	3231**	117**	2867
	SD	493	659	23	876
	% change	—	+67	−94	+48

From Buesen et al. (2009g)
cpm, counts per minute; SD, standard deviation; * $P < 0.05$; ** $P < 0.01$ (Wilcoxon test, two-sided)

Table 110. Thyroid weights and ^{125}I counts in female rats administered potassium perchlorate prior to sacrifice

		Group 01 ($n = 6$) Control + $KClO_4$	Group 11 ($n = 6$) Fluxapyroxad + $KClO_4$	Group 21 ($n = 6$) PTU + $KClO_4$	Group 31 ($n = 6$) PB + $KClO_4$
Thyroid weight (g)	Mean	0.009	0.010	0.041**	0.010
	SD	0.002	0.002	0.006	0.002
	% change	—	+11	+356	+11
Blood count (cpm)	Mean	5266	5593	9338**	6551*
	SD	462	1192	985	958
	% change	—	+6	+77	+24

		Group 01 (*n* = 6) Control + $KClO_4$	Group 11 (*n* = 6) Fluxapyroxad + $KClO_4$	Group 21 (*n* = 6) PTU + $KClO_4$	Group 31 (*n* = 6) PB + $KClO_4$
Thyroid count (cpm)	Mean	101 068	164 599*	87 107	155 325
	SD	41 125	45 492	29 636	48 609
	% change	—	+63	−14	+54
Specific blood count (cpm/g)	Mean	5172	5473	9081**	6365*
	% change	—	+6	+76	+23
Specific thyroid count (cpm/g)	Mean	10 848 075	16 795 840*	2 130 613**	14 816 334
	% change	—	+55	−80	+37
Ratio of specific thyroid/blood counts	Mean	2171	3221	234**	2460
	SD	891	1 022	61	925
	% change	—	+48	−89	+13

From Buesen et al. (2009g)
cpm, counts per minute; SD, standard deviation; * $P < 0.05$; ** $P < 0.01$ (Wilcoxon test, two-sided)

Administration of fluxapyroxad resulted in effects most similar in nature to those observed for PB. After 14 days of dietary exposure, no significant changes in thyroid weights were observed for males or females, but, similar to males treated with PB, a significant increase in the amount of ^{125}I in the thyroid was observed compared with control animals fed a normal diet. Also similar to males treated with PB, administration of perchlorate did not cause any changes in the amount of ^{125}I in the thyroid. No difference in ^{125}I thyroid content was observed for females exposed to fluxapyroxad relative to animals fed a normal diet. This was also similar to females treated with PB. Perchlorate treatment increased the amount of ^{125}I in the thyroid relative to controls, whereas it was unchanged for PB.

The similarities in the behaviour of fluxapyroxad to PB in this perchlorate discharge assay support the hypothesis that fluxapyroxad causes increases in thyroid hormones by an indirect mechanism (Buesen et al., 2009g).

The objective of the studies by Buesen et al. (2010b,c,d) was to investigate the effects of fluxapyroxad on hepatocyte proliferation in rats following dietary administration for various durations, to support the development of a proposed mechanism of action for possible effects on the liver and thyroid gland.

Groups of 10 male and 10 female Wistar rats were treated orally, by admixture in the diet, with fluxapyroxad (purity 99.2%) at a concentration of 0, 50, 250, 1500 or 3000 ppm (Table 111) for 1, 3, 7 and 14 days to investigate hepatocyte proliferation responses. Additionally, organ weight changes of liver and thyroid were determined and a histopathological evaluation of the liver was conducted.

Treatment with fluxapyroxad led to an increase in hepatocyte cell proliferation as early as 3 days after the start of treatment. The highest magnitude was observed in the centrilobular region (zone 3) of the liver. Cell proliferation responses were overall more pronounced in females than in males and were observed in males at 1500 and 3000 ppm and in females down to the lowest level of 50 ppm. Proliferative responses were increased after 3 days of treatment, reaching maximum levels after 7 days in males and females. After 14 days, cell proliferation was substantially lower in males, with levels slightly above control levels (Table 112). In females, cell proliferation was lower after 14 days of treatment, compared with maximum levels at day 7, but still significantly above controls at 250 ppm and higher (Table 113).

Table 111. Mean daily intake of fluxapyroxad by rats exposed for 1, 3, 7 or 14 days

Days	Mean daily intake (mg/kg bw per day)							
	50 ppm		250 ppm		1500 ppm		3000 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
1	3.0	3.6	15	17	86	91	150	146
3	3.0	3.2	16	15	93	82	176	186
7	3.3	3.5	16	17	100	92	183	195
14	4.0	3.5	17	20	106	104	201	214

From Buesen et al. (2010b)

Table 112. S-phase response in the liver of male rats (n = 10) administered fluxapyroxad for up to 14 days

Dietary concentration (ppm)	Mean S-phase response ± SD			
	Zone 1	Zone 2	Zone 3	All zones
14 days of treatment				
0	1.03 ± 0.34	3.86 ± 0.45	1.29 ± 0.28	2.07 ± 0.23
50	0.83 ± 0.40	3.57 ± 1.20	1.12 ± 0.51	1.87 ± 0.61
250	0.54 ± 0.28	3.13 ± 0.66	1.14 ± 0.39	1.62 ± 0.28
1500	0.55 ± 0.80	4.55 ± 2.78	2.71** ± 2.25	2.61 ± 1.78
3000	0.44 ± 0.28	3.59 ± 1.35	2.63** ± 1.52	2.25 ± 0.91
7 days of treatment				
50	0.23 ± 0.21	2.82 ± 0.96	0.62 ± 0.40	1.23 ± 0.45
250	0.33 ± 0.35	4.33 ± 1.51	0.60 ± 0.39	1.78 ± 0.71
1500	4.95** ± 2.17	19.51** ± 4.10	11.80** ± 3.98	11.70** ± 2.94
3000	7.43** ± 4.44	30.07** ± 9.45	19.09** ± 10.13	18.06** ± 7.13
3 days of treatment				
50	0.18 ± 0.19	2.41 ± 1.29	0.55 ± 0.33	4.05 ± 0.54
250	0.38 ± 0.31	3.03 ± 0.84	1.24 ± 0.56	1.58 ± 0.40
1500	4.05** ± 1.84	19.35** ± 8.68	9.36** ± 4.39	10.53** ± 4.43
3000	5.50** ± 2.45	28.32** ± 6.05	13.57** ± 4.26	15.16** ± 3.30
1 day of treatment				
50	0.52 ± 0.42	2.48 ± 0.70	1.02 ± 0.46	1.34 ± 0.44
250	0.58 ± 0.40	2.97 ± 1.30	0.83 ± 0.25	1.47 ± 0.60
1500	0.55 ± 0.28	3.88 ± 1.55	1.04 ± 0.56	1.84 ± 0.71
3000	0.48 ± 0.38	4.20 ± 0.90	1.15 ± 0.84	1.95 ± 0.57

From Buesen et al. (2010b)
SD, standard deviation; ** $P \leq 0.01$ (Wilcoxon test, one-sided)

Administration of fluxapyroxad resulted in dose- and time-dependent increases in absolute and/or relative liver weights, as early as 3 days after treatment (Table 114). Liver weight changes were observed at 1500 and 3000 ppm in both sexes at all time points, but also at 250 ppm in males after 14 days of treatment. The incidences and/or severity of enlarged liver and centrilobular hypertrophy were increased in males at 250 ppm and higher after 3, 7 and 14 days and in females at doses of 1500 and 3000 ppm after 7 and 14 days of treatment (Tables 115 and 116). No organ weight changes were observed in the thyroid gland (Buesen et al., 2010b).

Table 113. S-phase response in the liver of female rats (n = 10) administered fluxapyroxad for up to 14 days

Dietary concentration (ppm)	Mean S-phase response ± SD			
	Zone 1	Zone 2	Zone 1	All zones
14 days of treatment				
0	2.33 ± 1.12	1.23 ± 0.51	2.87 ± 1.39	2.14 ± 0.72
50	1.95 ± 0.91	1.17 ± 0.58	4.30 ± 2.44	2.47 ± 1.04
250	1.26 ± 0.78	1.00 ± 0.73	11.64** ± 2.00	4.63** ± 0.95
1500	1.08 ± 0.54	2.02* ± 0.99	25.17** ± 3.49	9.42** ± 1.40
3000	1.55 ± 1.12	4.54** ± 1.93	31.23** ± 6.37	12.44** ± 2.60
7 days of treatment				
50	2.82 ± 1.50	1.51 ± 1.26	7.79* ± 5.60	4.04* ± 2.30
250	3.60 ± 2.09	3.98** ± 3.55	16.63** ± 6.27	8.07** ± 3.66
1500	8.64** ± 3.97	11.60** ± 6.22	41.45** ± 8.34	20.56** ± 4.99
3000	15.17** ± 3.43	21.26** ± 5.73	44.62** ± 5.50	27.02** ± 4.28
3 days of treatment				
50	1.73 ± 0.95	0.91 ± 0.69	3.6 ± 1.81	2.08 ± 0.92
250	2.44 ± 1.21	1.38 ± 0.98	7.50** ± 3.52	3.77* ± 1.70
1500	5.88** ± 2.47	5.44** ± 2.08	22.68** ± 8.70	11.33** ± 3.82
3000	10.26** ± 4.97	12.61** ± 7.27	28.89** ± 9.14	17.25** ± 5.54
1 day of treatment				
50	2.06 ± 1.31	1.32 ± 0.48	3.75 ± 1.84	2.37 ± 0.92
250	1.89 ± 1.14	0.75 ± 0.40	3.28 ± 2.21	1.97 ± 0.89
1500	1.87 ± 0.88	0.95 ± 0.66	4.01 ± 2.98	2.28 ± 1.37
3000	2.86 ± 1.11	0.95 ± 0.52	3.44 ± 1.84	2.42 ± 0.99

From Buesen et al. (2010b)
SD, standard deviation; * $P \leq 0.05$; ** $P \leq 0.01$ (Wilcoxon test, one-sided)

Table 114. Liver and thyroid weights of rats after 1, 3, 7 and 14 days of treatment with fluxapyroxad

	Dietary concentration (ppm)	Mean weights ± SD							
		Males (*n* = 10)				Females (*n* = 10)			
		Absolute weight	% of control[a]	Relative weight[b]	% of control[a]	Absolute weight	% of control[a]	Relative weight[b]	% of control[a]
14 days of treatment									
Terminal weight (g)	0	336.8 ± 12.7	—	—	—	216.4 ± 16.0	—	—	—
	50	332.0 ± 16.4	(+99)	—	—	208.5 ± 9.4	(+96)	—	—
	250	329.7 ± 8.7	(+98)	—	—	213.3 ± 9.0	(+99)	—	—
	1500	339.3 ± 15.6	(+101)	—	—	208.4 ± 11.0	(+96)	—	—
	3000	328.6 ± 16.8	(+98)	—	—	214.7 ± 10.7	(+99)	—	—
Liver (g)	0	11.08 ± 1.05	—	3.285 ± 0.211	—	6.944 ± 0.824	—	3.201 ± 0.198	—

Table 114 (continued)

	Dietary concentration (ppm)	Mean weights ± SD							
		Males (*n* = 10)				Females (*n* = 10)			
		Absolute weight	% of control[a]	Relative weight[b]	% of control[a]	Absolute weight	% of control[a]	Relative weight[b]	% of control[a]
	50	11.18 ± 0.98	(+101)	3.363 ± 0.168	(+102)	6.763 ± 0.677	(+97)	3.242 ± 0.274	(+101)
	250	12.06* ± 0.75	(+109)	3.641* ± 0.259	(+111)	7.057 ± 0.562	(+102)	3.309 ± 0.231	(+103)
	1500	15.34** ± 0.96	(+138)	4.523** ± 0.239	(+138)	8.532** ± 0.639	(+123)	4.093** ± 0.178	(+128)
	3000	15.98** ± 1.51	(+144)	4.860** ± 0.348	(+148)	9.909** ± 0.706	(+143)	4.617** ± 0.259	(+144)
Thyroid (mg)	0	18.4	—	0.005 ± 0.001	—	17.6 ± 1.6	—	0.008 ± 0.001	—
	50	22.5* ± 4.1	(+122)	0.007* ± 0.001	(+124)	16.6 ± 1.8	(+94)	0.008 ± 0.001	(+98)
	250	23.5** ± 3.1	(+128)	0.007** ± 0.001	(+131)	18.5 ± 3.8	(+105)	0.009 ± 0.002	(+107)
	1500	25.5** ± 4.7	(+139)	0.008** ± 0.001	(+138)	17.9 ± 2.3	(+102)	0.009 ± 0.001	(+105)
	3000	24.5** ± 4.3	(+133)	0.007** ± 0.001	(+137)	18.8 ± 2.3	(+107)	0.009 ± 0.001	(+107)
7 days of treatment									
Terminal weight (g)	0	336.8 ± 12.7	—	—	—	216.4 ± 16.0	—	—	—
	50	336.6 ± 14.4	(+100)	—	—	218.4 ± 9.2	(+101)	—	—
	250	330.9 ± 18.0	(+98)	—	—	222.3 ± 12.7	(+103)	—	—
	1500	339.8 ± 15.2	(+101)	—	—	212.1± 12.1	(+98)	—	—
	3000	329.6 ± 2.9	(+98)	—	—	215.2 ± 10.1	(+99)	—	—
Liver (g)	0	11.08 ± 1.05	—	3.285 ± 0.211	—	6.944 ± 0.824	—	3.201 ± 0.198	—
	50	10.88 ± 0.69	(+98)	3.231 ± 0.154	(+98)	7.351 ± 0.655	(+106)	3.362 ± 0.215	(+105)
	250	11.42 ± 0.88	(+103)	3.447 ± 0.123	(+105)	7.638 ± 0.883	(+110)	3.432 ± 0.306	(+107)
	1500	14.11** ± 0.92	(+127)	4.152** ± 0.144	(+126)	8.259** ± 0.918	(+119)	3.887** ± 0.271	(+121)
	3000	14.88** ± 1.78	(+134)	4.502** ± 0.331	(+137)	9.067** ± 0.493	(+131)	4.214** ± 0.124	(+132)
Thyroid (mg)	0	18.4 ± 2.9	—	0.005 ± 0.001	—	17.6 ± 1.6	—	0.008 ± 0.001	—
	50	22.8* ± 3.4	(+124)	0.007** ± 0.001	(+124)	19.3 ± 2.5	(+110)	0.009 ± 0.001	(+108)
	250	23.6** ± 4.2	(+128)	0.007** ± 0.001	(+131)	19.0 ± 2.6	(+108)	0.009 ± 0.001	(+105)

	Dietary concentration (ppm)	Mean weights ± SD							
		Males (*n* = 10)				Females (*n* = 10)			
		Absolute weight	% of control[a]	Relative weight[b]	% of control[a]	Absolute weight	% of control[a]	Relative weight[b]	% of control[a]
	1500	23.4** ± 3.7	(+127)	0.007* ± 0.001	(+126)	18.5 ± 2.2	(+105)	0.009 ± 0.001	(+107)
	3000	23.5** ± 3.7	(+128)	0.007** ± 0.001	(+131)	18.9 ± 1.8	(+107)	0.009 ± 0.001	(+108)
3 days of treatment									
Terminal weight (g)	0	336.8 ± 12.7	—	—	—	216.4 ± 16.0	—	—	—
	50	337.0 ± 20.4	(+100)	—	—	218.0 ± 13.6	(+101)	—	—
	250	339.8 ± 14.0	(+101)	—	—	216.6 ± 7.4	(+100)	—	—
	1500	338.2 ± 14.6	(+100)	—	—	216.5 ± 11.8	(+100)	—	—
	3000	332.7 ± 22.4	(+99)	—	—	212.0 ± 10.6	(+98)	—	—
Liver (g)	0	11.08 ± 1.05	—	3.285 ± 0.211	—	6.944 ± 0.824	—	3.201 ± 0.198	—
	50	10.51 ± 1.12	(+95)	3.113 ± 0.162	(+95)	6.947 ± 0.669	(+100)	3.185 ± 0.195	(+99)
	250	11.76 ± 1.23	(+106)	3.454 ± 0.258	(+105)	7.077 ± 0.611	(+102)	3.266 ± 0.229	(+102)
	1500	13.29** ± 1.13	(+120)	3.927** ± 0.226	(+120)	7.911* ± 0.725	(+114)	3.654** ± 0.256	(+114)
	3000	13.74** ± 1.25	(+124)	4.126** ± 0.2	(+126)	8.855** ± 0.792	(+128)	4.178** ± 0.323	(+131)
Thyroid (mg)	0	18.4 ± 2.9	—	0.005 ± 0.001	—	17.6 ± 1.578	—	0.008 ± 0.001	—
	50	23.2** ± 3.9	(+126)	0.007* ± 0.001	(+126)	15.5 ± 3.0	(+88)	0.007 ± 0.001	(+87)
	250	23.4** ± 3.0	(+127)	0.007** ± 0.001	(+127)	16.9 ± 2.6	(+96)	0.008 ± 0.001	(+96)
	1500	23.6** ± 2.3	(+128)	0.007** ± 0.001	(+128)	16.7 ± 3.2	(+95)	0.008 ± 0.001	(+94)
	3000	21.1* ± 2.8	(+115)	0.006* ± 0.001	(+116)	15.9 ± 2.7	(+90)	0.007 ± 0.001	(+92)
1 day of treatment									
Terminal weight (g)	0	336.8 ± 12.7	—	—	—	216.4 ± 16.0	—	—	—
	50	344.1 ± 18.3	(+102)	—	—	218.1 ± 8.0	(+101)	—	—
	250	343.5 ± 19.0	(+102)	—	—	217.7 ± 9.5	(+101)	—	—
	1500	334.0 ± 15.0	(+99)	—	—	215.2 ± 11.5	(+99)	—	—
	3000	337.1 ± 12.5	(+100)	—	—	211.0 ± 8.9	(+98)	—	—

Table 114 (continued)

	Dietary concentration (ppm)	Mean weights ± SD							
		Males (n = 10)				Females (n = 10)			
		Absolute weight	% of control[a]	Relative weight[b]	% of control[a]	Absolute weight	% of control[a]	Relative weight[b]	% of control[a]
Liver (g)	0	11.08 ± 1.05	—	3.285 ± 0.211	—	6.944 ± 0.824	—	3.201 ± 0.198	—
	50	10.42 ± 0.91	(+94)	3.029* ± 0.208	(+92)	7.226 ± 0.529	(+104)	3.312 ± 0.197	(+103)
	250	10.90 ± 1.00	(+98)	3.172 ± 0.236	(+97)	6.965 ± 0.628	(+100)	3.195 ± 0.184	(+100)
	1500	10.53 ± 0.90	(+95)	3.148 ± 0.151	(+96)	7.017 ± 0.583	(+101)	3.263 ± 0.251	(+102)
	3000	11.18 ± 0.73	(+101)	3.311 ± 0.203	(+101)	6.577 ± 0.461	(+95)	3.115 ± 0.134	(+97)
Thyroid (mg)	0	18.4 ± 2.9	—	0.005 ± 0.001	—	17.6 ± 1.6	—	0.008 ± 0.001	—
	50	20.0 ± 3.2	(+109)	0.006 ± 0.211	(+107)	19.2 ± 1.8	(+109)	0.009 ± 0.001	(+108)
	250	21.6 ± 2.1	(+117)	0.006 ± 0.001	(+115)	17.3 ± 2.7	(+98)	0.008 ± 0.001	(+97)
	1500	21.6 ± 3.1	(+117)	0.006 ± 0.001	(+119)	17.8 ± 2.5	(+101)	0.008 ± 0.001	(+101)
	3000	21.7 ± 2.9	(+118)	0.006 ± 0.001	(+118)	18.7 ± 3.1	(+106)	0.009 ± 0.002	(+109)

From Buesen et al. (2010b)
SD, standard deviation; * $P < 0.05$; ** $P < 0.01$ (Kruskal-Wallis test, two-sided)
[a] Compared with control. Values may not calculate exactly due to rounding.
[b] Percentage of body weight.

Table 115. Incidence of enlarged liver in rats administered fluxapyroxad for up to 14 days

	Incidence of enlarged liver							
	Males (n = 10)				Females (n = 10)			
	0 ppm	250 ppm	1500 ppm	3000 ppm	0 ppm	250 ppm	1500 ppm	3000 ppm
14 days of treatment	0	0	10	10	0	0	5	10
7 days of treatment	0	0	10	10	0	0	4	10
3 days of treatment	0	0	6	7	0	0	2	7
1 day of treatment	0	0	0	0	0	0	0	0

From Buesen et al. (2010b)

Groups of 10 male and 10 female Wistar rats were treated orally, by admixture in the diet, with fluxapyroxad (purity 99.2%) at a concentration of 0, 250, 1500 or 3000 ppm (Table 117) for 7, 28 and 91 days to investigate hepatocyte proliferation responses. The reversibility of fluxapyroxad's effects on cell proliferation was determined after treatment with 3000 ppm fluxapyroxad for 28 days followed by a 28-day withdrawal period. Additionally, changes in liver and thyroid weights were measured and a histopathological evaluation of the liver was conducted.

Table 116. Incidence of centrilobular hypertrophy of liver in rats administered fluxapyroxad for up to 14 days

	Incidence and severity[a] of finding							
	Males (n = 10)				Females (n = 10)			
	0 ppm ppm	250	1500 ppm	3000 ppm	0 ppm	250 ppm	1500 ppm	3000 ppm
14 days of treatment	0	2 [2.0]	10 [2.5]	7 [2.3]	0	0	10 [2.5]	10 [3.0]
7 days of treatment	0	1 [1.0]	7 [2.3]	4 [2.0]	0	0	0	4 [2.3]
3 days of treatment	0	0	0	0	0	0	0	8 [2.0]
1 day of treatment	0	0	0	0	0	0	0	0

From Buesen et al. (2010b)

[a] [ ] mean severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked (Grade 4) and massive/severe (Grade 5). The mean severity is the sum of the gradings divided by the incidence.

Table 117. Mean daily intake of fluxapyroxad by rats exposed for 7, 28 or 91 days

Exposure duration (days)	Mean daily intake (mg/kg bw per day)							
	250 ppm		1500 ppm		3000 ppm		3000 ppm recovery group	
	Males	Females	Males	Females	Males	Females	Males	Females
7	12	15	61	79	104	137	NA	NA
28	12	15	79	87	122	173	131	172
91	13	17	80	106	163	190	NA	NA

From Buesen et al. (2010c)

NA, not applicable

No clinical signs were observed, with the exception of tooth whitening (Table 118). This effect was noted after 91 days of treatment and was consistent with findings in other toxicity studies with fluxapyroxad. Body weight gain was decreased in the 3000 ppm group females, beginning on day 56; no effect on body weight was observed in males (Tables 119, 120 and 121).

Table 118. Incidence of abnormal teeth whitening after 91 days of treatment with fluxapyroxad

Dietary concentration (ppm)	Incidence of abnormal teeth whitening			
	Males (n = 10)		Females (n = 10)	
	Lower jaw	Upper jaw	Lower jaw	Upper jaw
0	0	0	0	0
250	0	0	0	0
1500	1	0	6	0
3000	4	0	10	0

From Buesen et al. (2010c)

Table 119. Mean body weight and body weight gain of rats administered fluxapyroxad for 91 days (treatment from day 0 to day 91)

	Mean value ± SD							
	Males (n = 10)				Females (n = 10)			
	0 ppm	250 ppm	1500 ppm	3000 ppm	0 ppm	250 ppm	1500 ppm	3000 ppm
Body weight (g)								
- day 0	292.5 ± 11.5	291.4 ± 13.2	292.1 ± 12.9	291.2 ± 10.5	198.6 ± 3.0	196.6 ± 8.1	196.5 ± 6.4	196.5 ± 6.2
- day 91	444.9 ± 26.4	441.9 ± 24.9	440.8 ± 27.6	428.0 ± 32.8	258.4 ± 8.9	248.5 ± 9.2	229.9 ± 10.1	240.9** ±11.7 (−6.8%)
% change[a]	—	*−0.7*	*−0.9*	*−3.8*	—	*−3.8*	*−4.8*	*−6.8*
Overall body weight gain (g)[b]	152.4	150.5	148.7	136.7	59.8	51.9	49.4	44.4
% change[a]	—	*−1.3*	*−2.4*	*−10.3*	—	*−13.2*	*−17.4*	*−25.8*

From Buesen et al. (2010c)
SD, standard deviation; ** $P \leq 0.05$ (Dunnett's test, two-sided)
[a] Compared with control.
[b] No standard deviations reported.

Table 120. Mean body weight and body weight gain of rats administered fluxapyroxad for 28 days (treatment from day 63 to day 91, recovery from day 91 to day 119)

	Mean value ± SD									
	Males (n = 10)					Females (n = 10)				
	0 ppm	250 ppm	1500 ppm	3000 ppm	3000 ppmR[a]	0 ppm	250 ppm	1500 ppm	3000 ppm	3000 ppmR[a]
Body weight (g)										
- day 0	294.6 ± 11.8	291.6 ± 10.0	295.1 ± 8.2	294.9 ± 10.8	294.6 ± 12.8	191.4 ± 5.8	193.5 ± 9.0	191.1 ± 7.6	192.6 ± 8.2	191.7 ± 5.5
- day 91	435.0 ± 32.5	439.8 ± 27.8	447.2 ± 27.0	437.7 ± 26.7	407.4 ± 44.8	250.3 ± 15.7	260.1 ± 18.1	245.6 ± 17.9	247.3 ± 19.1	240.1 ± 8.6
- day 119	460.8 ± 34.9	—	—	—	434.3 ± 42.3	262.2 ± 16.6	—	—	—	253.5 ± 10.6
% change[b]										
- end of treatment	—	+1.1	+2.8	+0.6	−6.3	—	+3.9	−1.9	−1.2	−4.1
- end of recovery	—	—	—	—	−5.8	—	—	—	—	−3.3
Overall body weight gain (g)										
- end of treatment[c]	140.3	148.3	152.1	142.8	112.9	58.9	66.6	54.5	54.8	48.4
- end of recovery	166.2	—	—	—	139.7	70.8	—	—	—	61.8
% change[b]										
- end of treatment	—	+5.7	+8.4	+1.8	−19.6	—	+13.1	−7.5	−7.0	−17.9
- end of recovery	—	—	—	—	−15.9	—	—	—	—	−12.7

From Buesen et al. (2010c)
SD, standard deviation
[a] R = recovery group, 28-day treatment followed by a withdrawal period of 28 days.
[b] Compared with control.
[c] No standard deviations reported.

Table 121. Mean body weight of rats administered fluxapyroxad for 7 days (treatment from day 84 to day 91)

	Mean value ± SD							
	Males (n = 10)				Females (n = 10)			
	0 ppm	250 ppm	1500 ppm	3000 ppm	0 ppm	250 ppm	1500 ppm	3000 ppm
Body weight (g)								
- day 0	292.5 ± 11.5	297.4 ± 12.6	277.2* ± 19.5 (−5.2%)	288.1 ± 9.8	198.6 ± 3.0	198.7 ± 9.8	195.4 ± 5.9	198.6 ± 12.6
- day 91	444.9 ± 26.4	443.4 ± 30.6	451.7 ± 21.3	436.5 ± 36.2	258.4 ± 8.9	255.0 ± 10.1	245.7* ± 9.8 (−4.9%)	246.7 ± 14.4
% change[a]	—	*−0.3*	*+1.5*	*−1.9*	—	*−1.3*	*−4.9**	*−4.5*
Overall body weight gain (g)[b]	152.4	147.7	174.4	148.4	59.8	56.3	50.3	48.2
% change[a]	—	*−3.1*	*+14.5*	*−2.6*	—	*−5.9*	*−15.8*	*−19.4**

From Buesen et al. (2010c)
SD, standard deviation; * $P \leq 0.05$ (Dunnett's test, two-sided)
[a] Compared with control.
[b] No standard deviations reported.

Treatment with fluxapyroxad led to an increase in hepatocyte cell proliferation as early as 7 days after the start of treatment. The highest magnitude was observed in the centrilobular region (zone 3) of the liver. Cell proliferation responses were more pronounced in females than in males; in males, they were observed at 1500 and 3000 ppm (Table 122), whereas in females, they occurred down to the lowest dose level of 250 ppm. Proliferative responses were highest after 7 days of treatment in males and females (Table 123). After 28 and 91 days, cell proliferation was substantially lower in males, with levels close to, or slightly above, control levels. In females, cell proliferation was lower after 28 and 91 days of treatment, compared with maximum levels at day 7, but was still significantly above that of controls at all dose levels. No increase in cell proliferation was noted after withdrawal of treatment, indicating reversibility of the effects.

Table 122. S-phase response in the liver of male rats

Dietary concentration (ppm)	Labelling index ± SD			
	Zone 1	Zone 2	Zone 3	All zones
91 days of treatment (n = 10)				
0	0.98 ± 0.36	0.74 ± 0.39	0.72 ± 0.23	0.81 ± 0.24
250	0.64 ± 0.25	0.62 ± 0.32	0.79 ± 0.63	0.68 ± 0.31
1500	0.69 ± 0.42	0.58 ± 0.31	1.06 ± 0.59	0.78 ± 0.27
3000	0.72 ± 0.28	0.94 ± 0.38	1.99** ± 1.35	1.22 ± 0.52
28 days of treatment (n = 10)				
250	0.71 ± 0.27	0.50 ± 0.31	0.68 ± 0.44	0.63 ± 0.19
1500	0.75 ± 0.53	0.47 ± 0.38	0.92 ± 0.84	0.71 ± 0.50
3000	0.72 ± 0.39	0.65 ± 0.31	1.28 ± 1.13	0.88 ± 0.44
7 days of treatment (n = 10)				
250	0.84 ± 0.42	0.57 ± 0.49	0.84 ± 0.55	0.75 ± 0.41
1500	4.27** ± 1.41	5.42** ± 1.39	9.96** ± 2.67	6.55** ± 1.62

Table 122 (continued)

Dietary concentration (ppm)	Labelling index ± SD			
	Zone 1	Zone 2	Zone 3	All zones
3000	2.98** ± 1.66	6.16** ± 4.27	15.23** ± 8.00	8.12** ± 4.16
28 days of treatment and 28 days of recovery (*n* = 10)				
0	0.79 ± 0.36	0.56 ± 0.36	0.52 ± 0.31	0.62 ± 0.28
3000	0.42 ± 0.22	0.17 ± 0.19	0.15 ± 0.14	0.25 ± 0.15

From Buesen et al. (2010c)
SD, standard deviation; * $P \leq 0.05$; ** $P \leq 0.01$ (Wilcoxon test, one-sided)

Table 123. S-phase response in the liver of female rats

Dietary concentration (ppm)	Labelling index ± SD			
	Zone 1	Zone 2	Zone 1	All zones
91 days of treatment (*n* = 10)				
0	2.48 ± 0.74	0.97 ± 0.81	1.11 ± 1.32	1.52 ± 0.85
250	1.32 ± 0.46	0.87 ± 0.56	2.98** ± 1.12	1.72 ± 0.56
1500	0.53 ± 0.26	1.05 ± 0.31	6.00** ± 1.99	2.53** ± 0.62
3000	0.30 ± 0.23	0.82 ± 0.56	7.89** ± 2.56	3.00** ± 0.81
28 days of treatment (*n* = 10)				
250	1.09 ± 0.66	1.13 ± 0.78	7.77** ± 7.21	3.33** ± 2.69
1500	0.24 ± 0.12	0.59 ± 0.24	13.58** ± 5.21	4.80** ± 1.72
3000	0.45 ± 0.25	1.55* ± 0.72	15.18** ± 3.92	5.73** ± 1.32
7 days of treatment (*n* = 10)				
250	2.92 ± 0.95	1.62* ± 0.77	4.82** ± 2.34	3.12** ± 0.89
1500	5.51** ± 2.26	7.12** ± 2.54	25.30** ± 5.95	12.64** ± 2.74
3000	13.88** ± 4.25	15.74** ± 7.19	28.47** ± 7.32	19.36** ± 5.01
28 days of treatment and 28 days of recovery (*n* = 10)				
0	1.76 ± 0.62	0.42 ± 0.19	0.47 ± 0.29	0.89 ± 0.27
3000	0.14 ± 0.07	0.03 ± 0.07	0.05 ± 0.09	0.07 ± 0.05

From Buesen et al. (2010c)
SD, standard deviation; * $P \leq 0.05$; ** $P \leq 0.01$ (Wilcoxon test, one-sided)

Administration of fluxapyroxad resulted in dose- and time-dependent increases in absolute and/or relative liver weights (Tables 124 and 125) and centrilobular hypertrophy as early as 7 days after treatment. Liver weight changes were observed at 1500 and 3000 ppm, and incidences and/or severity of centrilobular hypertrophy and liver enlargement were increased at all dose levels in both sexes (Tables 126 and 127). No liver weight or centrilobular hypertrophy changes were observed following withdrawal of treatment. No weight changes were observed in the thyroid glands.

Table 124. Body and organ weights after 7, 28 and 91 days of treatment with fluxapyroxad

	Dietary concentration (ppm)	Mean value ± SD							
		Males (*n* = 10)				Females (*n* = 10)			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
91 days of treatment									
Terminal weight (g)	0	440.8 ± 26.7	—	—	—	253.2 ± 7.9	—	—	—
	250	436.6 ± 24.3	(+99)	—	—	241.5* ± 11.0	(+95)	—	—
	1500	435.1 ± 29.1	(+99)	—	—	239.3** ± 10.4	(+95)	—	—
	3000	412.0 ± 23.9	(+93)	—	—	233.7** ± 12.0	(+92)	—	—
Liver (g)	0	11.44 ± 0.90	—	2.597 ± 0.175	—	7.184 ± 0.568	—	2.837 ± 0.208	—
	250	11.85 ± 1.01	(+104)	2.718 ± 0.235	(+105)	7.174 ± 0.518	(+100)	2.969 ± 0.12	(+105)
	1500	14.83** ± 1.39	(+130)	3.405** ± 0.128	(+131)	8.766** ± 0.683	(+122)	3.664** ± 0.255	(+129)
	3000	16.11** ± 1.54	(+141)	3.828* ± 0.177*	(+147)	9.607** ± 0.514	(+134)	4.114** ± 0.197	(+145)
Thyroid (mg)	0	31.8 ± 8.3	—	0.007 ± 0.002	—	21.5 ± 3.4	—	0.009 ± 0.001	—
	250	29.9 ± 4.7	(+94)	0.007 ± 0.001	(+95)	22.8 ± 2.8	(+106)	0.009 ± 0.001	(+111)
	1500	30.7 ± 3.9	(+97)	0.007 ± 0.001	(+98)	26.1 ± 4.8	(+121)	0.011** ± 0.002	(+128)
	3000	28.1 ± 2.6	(+88)	0.007 ± 0.001	(+94)	25.0 ± 4.4	(+116)	0.011** ± 0.002	(+126)
28 days of treatment									
Terminal weight (g)	0	440.8 ± 26.7	—	—	—	253.2 ± 7.9	—	—	—
	250	436.0 ± 27.0	(+99)	—	—	255.6 ± 17.9	(+101)	—	—
	1500	441.7 ± 27.5	(+100)	—	—	241.0 ± 17.6	(+95)	—	—
	3000	433.0 ± 24.9	(+98)	—	—	242.4 ± 18.8	(+96)	—	—
Liver (g)	0	11.44 ± 0.90	—	2.597 ± 0.175	—	7.184 ± 0.568	—	2.837 ± 0.208	—
	250	11.78 ± 1.13	(+103)	2.702 ± 0.183	(+104)	7.694 ± 0.869	(+107)	3.016 ± 0.339	(+106)
	1500	15.13** ± 1.04	(+132)	3.431** ± 0.25	(+132)	7.893 ± 0.77	(+110)	3.273** ± 0.185	(+115)
	3000	16.28** ± 1.56	(+142)	3.758** ± 0.212	(+145)	9.269** ± 0.767	(+129)	3.828** ± 0.203	(+135)
Thyroid (mg)	0	31.8 ± 8.3	—	0.007 ± 0.002	—	21.5 ± 3.4	—	0.009 ± 0.001	—

Table 124 (continued)

	Dietary concentration (ppm)	Mean value ± SD							
		Males (*n* = 10)				Females (*n* = 10)			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
	250	23.4* ± 5.6	(+74)	0.005* ± 0.001	(+75)	23.8 ± 1.5	(+111)	0.009 ± 0.001	(+110)
	1500	25.3* ± 3.5	(+80)	0.006 ± 0.001	(+80)	24.8 ± 3.9	(+115)	0.01 ± 0.002	(+122)
	3000	27.7 ± 3.5	(+87)	0.006 ± 0.001	(+89)	24.0 ± 3.9	(+112)	0.01 ± 0.002	(+118)
7 days of treatment									
Terminal weight (g)	0	440.8 ± 26.7	—	—	—	253.2 ± 8.0	—	—	—
	250	437.6 ± 31.1	(+99)	—	—	250.3 ± 9.0	(+99)	—	—
	1500	445.2 ± 20.7	(+101)	—	—	241.2* ± 9.5	(+95)	—	—
	3000	431.0 ± 36.4	(+98)	—	—	239.6* ± 15.3	(+95)	—	—
Liver (g)	0	11.44 ± 0.90	—	2.597 ± 0.175	—	7.184 ± 0.568	—	2.837 ± 0.208	—
	250	11.7 ± 1.5	(+102)	2.664 ± 0.193	(+103)	7.108 ± 0.692	(+99)	2.84 ± 0.21	(+100)
	1500	13.88** ± 0.96	(+121)	3.12** ± 0.22	(+120)	7.739 ± 0.852	(+108)	3.206** ± 0.289	(+113)
	3000	14.59** ± 1.77	(+128)	3.382** ± 0.242	(+130)	8.008** ± 0.448	(+111)	3.347** ± 0.144	(+118)
Thyroid (mg)	0	31.8 ± 8.3	—	0.007 ± 0.002	—	21.5 ± 3.44	—	0.009 ± 0.001	—
	250	24.7* ± 3.1	(+78)	0.006 ± 0.001	(+78)	15.2** ± 3.6	(+71)	0.006** ± 0.001	(+72)
	1500	24.4* ± 3.5	(+77)	0.005 ± 0.001	(+76)	20.5 ± 3.1	(+95)	0.009 ± 0.001	(+100)
	3000	22.7** ± 3.7	(+71)	0.005 ± 0.001	(+74)	19.0 ± 1.9	(+88)	0.008 ± 0.001	(+93)

From Buesen et al. (2010c)

SD, standard deviation; * $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)

[a] Compared with controls. Values may not calculate exactly due to rounding.

[b] Percentage of body weight.

Table 125. Body and organ weights after 28 days of treatment followed by a 28-day recovery period

	Dietary concentration (ppm)	Mean value ± SD							
		Males (*n* = 10/group)				Females (*n* = 10/group)			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
Terminal weight (g)	0	460.8 ± 36.2	—	—	—	258.1 ± 16.6	—	—	—
	250	—	—	—	—	—	—	—	—

	Dietary concentration (ppm)	Mean value ± SD							
		Males ($n = 10$/group)				Females ($n = 10$/group)			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
	1500	—	—	—	—	—	—	—	—
	3000	435.7 ± 44.3	(+95)	—	—	249.2 ± 9.9	(+97)	—	—
Liver (g)	0	12.33 ± 1.40	—	2.674 ± 0.185	—	7.146 ± 0.621	—	2.769 ± 0.162	—
	250	—	—	—	—	—	—	—	—
	1500	—	—	—	—	—	—	—	—
	3000	12.10 ± 1.43	(+98)	2.777 ± 0.161	(+104)	7.644 ± 0.655	(+107)	3.064** ± 0.189	(+111)
Thyroid (mg)	0	23.8 ± 4.4	—	0.005 ± 0.001	—	22.8 ± 3.676	—	0.009 ± 0.001	—
	250	—	—	—	—	—	—	—	—
	1500	—	—	—	—	—	—	—	—
	3000	20.0 ± 2.9	(+84)	0.005 ± 0.001	(+89)	20.3 ± 2.7	(+89)	0.008 ± 0.001	(+92)

From Buesen et al. (2010c)

SD, standard deviation; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)

[a] Compared with control. Values may not calculate exactly due to rounding.

[b] Percentage of body weight.

Table 126. Incidence of selected gross pathological findings in rats administered fluxapyroxad for up to 91 days

	Incidence of finding							
	Males ($n = 10$/group)				Females ($n = 10$/group)			
	0 ppm	250 ppm	1500 ppm	3000 ppm	0 ppm	250 ppm	1500 ppm	3000 ppm
91 days of treatment								
Incisor								
Mandible, discoloration	0	0	1	4	0	0	6	10
Maxilla, discoloration	0	0	0	0	0	0	0	10
Liver								
Discoloration	0	0	0	0	0	0	3	10
Enlarged	0	0	3	9	0	0	4	8
28 days of treatment								
Liver								
Enlarged	0	0	8	10	0	0	0	10
7 days of treatment								
Liver								
Enlarged	0	0	0	5	0	0	0	0
28 days of treatment and 28 days of recovery								
Liver								
Enlarged	0	0	0	2	0	0	0	0

From Buesen et al. (2010c)

Table 127. Incidence of centrilobular hypertrophy of liver in rats administered fluxapyroxad for up to 91 days

	Incidence and severity[a] of finding							
	Males (n = 10/group)				Females (n = 10/group)			
	0 ppm	250 ppm	1500 ppm	3000 ppm	0 ppm	250 ppm	1500 ppm	3000 ppm
91 days of treatment	—	5	10	10	—	5	10	10
		[1.0]	[3.2]	[3.4]		[1.2]	[3.0]	[4.0]
28 days of treatment	—	9	10	10	—	4	10	10
		[1.4]	[2.2]	[2.9]		[1.0]	[2.6]	[3.8]
7 days of treatment	—	4	10	10	—	1	9	10
		[1.3]	[2.0]	[2.1]		[1.0]	[1.7]	[1.8]
28 days of treatment and 28 days of recovery	—	—	—	0	—	—	—	0

From Buesen et al. (2010c)

[a] [ ] mean severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked (Grade 4) and massive/severe (Grade 5). The mean severity is the sum of the gradings divided by the incidence.

The cell proliferation responses in the liver of Wistar rats following administration of fluxapyroxad and reversibility following withdrawal were consistent with a mitogenic mode of action (Buesen et al., 2010c).

Groups of 10 male and 10 female Wistar rats were treated orally, by admixture in the diet, with fluxapyroxad (purity 99.2%) at a concentration of 0 or 50 ppm (Table 128) for 7, 28 and 91 days to investigate hepatocyte proliferation responses. Additionally, weight changes of liver and thyroid were measured, a histopathological evaluation of the liver was performed and the reversibility of these effects was determined.

Table 128. Mean daily intake of fluxapyroxad by rats exposed for 7, 28 or 91 days

Treatment period (days)	Dietary concentration (ppm)	Mean daily test item intake (mg/kg bw per day)	
		Males	Females
7	50	2.5	2.9
28	50	2.5	3.1
91	50	3.0	3.5

From Buesen et al. (2010d)

There were no mortalities or clinical signs and no effects on body weight gain, feed consumption or organ weights. No gross pathological or histopathological changes were observed. Treatment with 50 ppm fluxapyroxad for 7, 28 and 91 days had no effect on hepatocyte cell proliferation in males or females (Tables 129 and 130). Consistently, administration of fluxapyroxad resulted in no organ weight changes or histopathological findings (Buesen et al., 2010d).

This cell proliferation study was conducted as a complement to a previous study (Buesen et al., 2010c), to include investigation of all dose levels tested in the 2-year rat study.

Table 129. S-phase response in the liver of male rats

Dietary concentration (ppm)	Labelling index ± SD			
	Zone 1	Zone 2	Zone 3	All zones
91 days of treatment (*n* = 10)				
0	0.81 ± 0.42	0.55 ± 0.31	0.91 ± 0.41	0.76 ± 0.23
50	0.61 ± 0.30	0.77 ± 0.39	0.80 ± 0.68	0.73 ± 0.30
28 days of treatment (*n* = 10)				
50	0.78 ± 0.44	0.57 ± 0.41	0.60 ± 0.44	0.65 ± 0.41
7 days of treatment (*n* = 10)				
50	0.93 ± 0.41	0.84 ± 0.49	0.72 ± 0.41	0.83 ± 0.31

From Buesen et al. (2010d)
SD, standard deviation

Table 130. S-phase response in the liver of female rats

Dietary concentration (ppm)	Labelling index ± SD			
	Zone 1	Zone 2	Zone 3	All zones
91 days of treatment (*n* = 10)				
0	1.88 ± 0.56	0.43 ± 0.16	0.51 ± 0.25	0.94 ± 0.20
50	1.55 ± 0.71	0.50 ± 0.35	0.53 ± 0.32	0.86 ± 0.25
28 days of treatment (*n* = 10)				
50	2.55* ± 0.81	0.80** ± 0.35	0.76 ± 0.55	1.37* ± 0.46
7 days of treatment (*n* = 10)				
50	1.54 ± 0.63	0.68 ± 0.44	0.79 ± 0.55	1.01* ± 0.51

From Buesen et al. (2010d)
SD, standard deviation; * $P < 0.05$; ** $P < 0.01$ (Wilcoxon test)

(b) Neurotoxicity

In an acute neurotoxicity study, fluxapyroxad (purity 99.4%) was administered to young adult Wistar (Crl:WI(Han)) rats (10 of each sex per dose) via gavage in 10 ml/kg bw of an aqueous suspension (1% aqueous carboxymethylcellulose) at a dose level of 0, 125, 500 or 2000 mg/kg bw and then maintained for a 14-day observation period. Feed consumption and body weights were recorded, and a functional observational battery of tests, including a quantitative assessment of motor activity, was performed on all animals predosing, on the day of treatment and on days 7 and 14. Five animals of each sex per dose were subjected to necropsy, postmortem examination, brain weight and dimensions recording, perfusion fixation and preservation of brain, dorsal root fibres and ganglia, ventral root fibres, spinal cord, eyes, optic, tibial and sciatic nerves, skeletal muscle and gross lesions.

No premature deaths occurred, and there were no adverse clinical signs at the routine observation intervals. There was no effect on body weight gain (Table 131). Treatment-related neurobehavioural effects were noted in mid- and high-dose animals only on the day of treatment. These consisted of slight, but statistically significant, increase of the landing foot splay in high-dose males (Table 132), reduction in the number of rearings and exploration in males at and above 500 mg/kg bw per day (Tables 133 and 134) as well as impaired motor activity in high- and mid-dose males and females (Table 135). No effects on these parameters were observed on study days 7 and 14. Additionally, no treatment-related neuropathological findings were noted—that is, no brain weight changes or neurohistopathological findings were observed (Tables 136 and 137). Therefore, the affected clinical parameters were considered an indication of a neuropharmacological effect rather than an indication of neuronal damage.

Table 131. Mean body weights and body weight gain of rats administered fluxapyroxad once and observed for 14 days

	Mean value ± SD							
	Males (n = 10/group)				Females (n = 10/group)			
	0 mg/kg bw	125 mg/kg bw	500 mg/kg bw	2000 mg/kg bw	0 mg/kg bw	125 mg/kg bw	500 mg/kg bw	2000 mg/kg bw
Body weight (g)								
- day 0	231.2 ± 7.1	232.0 ± 7.9	231.5 ± 7.1	228.7 ± 5.8	175.5 ± 5.2	174.7 ± 9.0	170.9 ± 7.6	174.1 ± 6.3
- day 14	296.6 ± 9.8	301.8 ± 14.3	295.2 ± 14.4	294.6 ± 10.7	200.5 ± 8.8	206.6 ± 9.3	198.0 ± 9.2	200.0 ± 7.2
% change[a]	—	*+1.8*	*−0.5*	*−0.7*	—	*+3.1*	*−1.3*	*−0.3*
Overall body weight gain (g)	65.4 ± 9.0	69.8 ± 7.9	63.8 ± 9.1	65.9 ± 5.7	24.9 ± 5,6	31.9* ± 7.1	27.1 ± 4.2	25.8 ± 6.5
% change[a]	—	*+6.7*	*−2.6*	*+0.7*	—	*+28.0*	*+8.7*	*+3.6*

From Kaspers et al. (2009b)

SD, standard deviation; * $P < 0.05$ (Dunnett's test, two-sided)

[a] Compared with control. Values may not calculate exactly due to rounding of mean values (values given in the table are based on unrounded means).

Table 132. Foot splay values in rats administered fluxapyroxad once and observed for 14 days

	Foot splay (cm)			
	0 mg/kg bw	125 mg/kg bw	500 mg/kg bw	2000 mg/kg bw
Males (*n* = 10)				
Day −7	9.4 ± 0.7	9.6 ± 1.1	10.3 ± 1.2	10.4 ± 0.8
Day 0	10.4 ± 1.1	11.1 ± 1.1	11.5 ± 1.3	11.9** ± 1.1
Day 7	10.5 ± 1.3	10.4 ± 1.0	11.2 ± 2.1	10.8 ± 0.9
Day 14	13.1 ± 1.5	13.4 ± 1.9	14.1 ± 2.1	13.0 ± 1.4
Females (*n* = 10)				
Day −7	8.7 ± 1.0	9.4 ± 1.4	9.4 ± 1.5	9.5 ± 1.6
Day 0	10.0 ± 1.7	9.8 ± 1.2	10.2 ± 1.2	10.4 ± 1.7
Day 7	9.5 ± 1.3	9.5 ± 1.0	9.5 ± 0.9	9.6 ± 1.0
Day 14	11.1 ± 1.7	11.0 ± 2.1	10.6 ± 1.2	10.9 ± 1.9

From Kaspers et al. (2009b)

** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)

Table 133. Number of rearings in rats administered fluxapyroxad once and observed for 14 days

	Number of rearings			
	0 mg/kg bw	125 mg/kg bw	500 mg/kg bw	2000 mg/kg bw
Males (*n* = 10)				
Day −7	6.5 ± 2.6	6.1 ± 4.4	6.4 ± 5.4	6.9 ± 4.6
Day 0	2.8 ± 2.9	1.4 ± 1.6	0.2** ± 0.6 (−93%)	0.2** ± 0.4 (−93%)
Day 7	2.2 ± 2.4	3.5 ± 1.7	2.0 ± 2.4	1.6 ± 1.3
Day 14	3.7 ± 3.1	5.4 ± 3.9	3.6 ± 3.1	2.5 ± 3.5

	Number of rearings			
	0 mg/kg bw	125 mg/kg bw	500 mg/kg bw	2000 mg/kg bw
Females (n = 10)				
Day −7	10.6 ± 3.4	8.3 ± 5.5	12.6 ± 4.0	8.3 ± 5.3
Day 0	4.6 ± 5.1	3.0 ± 4.0	2.6 ± 3.0	2.9 ± 4.5
Day 7	6.6 ± 3.4	8.6 ± 5.3	12.7 ± 6.7	9.7 ± 5.7
Day 14	6.3 ± 4.5	7.7 ± 5.4	8.1 ± 5.5	9.8 ± 6.5

From Kaspers et al. (2009b)
** $P \leq 0.01$ (Kruskal-Wallis + Wilcoxon test, two-sided)

Table 134. Incidence of the open-field observation "reduced exploration of area" in rats administered fluxapyroxad once and observed for 14 days

	Incidence of open-field observation "activity/arousal level" rank 1: reduced exploration of the area							
	Males				Females			
	0 mg/kg bw	125 mg/kg bw	500 mg/kg bw	2000 mg/kg bw	0 mg/kg bw	125 mg/kg bw	500 mg/kg bw	2000 mg/kg bw
Day −7	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Day 0	0/10	5/10	9/10	7/10	2/10	5/10	2/10	2/10
Day 7	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Day 14	0/10	0/10	0/10	0/10	2/10	2/10	1/10	0/10

From Kaspers et al. (2009b)

Table 135. Overall mean motor activity in rats administered fluxapyroxad once and observed for 14 days

	Mean number of beam interruptions			
	0 mg/kg bw	125 mg/kg bw	500 mg/kg bw	2000 mg/kg bw
Males (n = 10)				
Day −7, total	3739.8 ± 1443.8	3645.9 ± 1239.5	3443.6 ± 932.9	3365.1 ± 963.6
Day 0, total	3760.0 ± 927.5	3609.9 ± 702.0	2477.5 ** ± 620.3 (−34%)	1696.6** ± 372.2 (−55%)
Interval 1	1240 ± 384.0	1099 ± 235.7	944.6* ± 224.9 (−24%)	829.9* ± 271.1 (−33%)
Interval 2	1040 ± 323.7	961.6 ± 396.9	790.3* ± 218.8 (−24%)	439.0** ± 103.4 (−58%)
Interval 3	741.1 ± 30.4	613.1 ± 248.6	423.9** ± 192.2 (−43%)	166.7** ± 384.0 (−78%)
Interval 4	347.1 ± 205.9	291.4 ± 138.3	121.3* ± 113.7 (−65%)	30.1** ± 55.8 (−91%)
Interval 5	142.7 ± 100.1	229.7 ± 127.6	34.8** ± 63.7 (−76%)	14.0** ± 18.8 (−90%)
Day 7, total	4658.6 ± 1063.6	4549.6 ± 1135.3	3784.3 ± 738.3	4362.9 ± 923.8
Day 14, total	4185.6 ± 1203.5	4510.3 ± 1269.1	4153.5 ± 781.6	4950.9 ± 1242.5
Females (n = 10)				
Day −7, total	4201.1 ± 1356.3	4285.4 ± 1172.3	3842.3 ± 1285.2	4457.1 ± 909.7
Day 0, total	4789.0 ± 1305.6	3656.4 ± 1661.6	2936.4* ± 1374.8 (−39%)	2072.3** ± 696.9 (−57%)

Table 135 (continued)

	Mean number of beam interruptions			
	0 mg/kg bw	125 mg/kg bw	500 mg/kg bw	2000 mg/kg bw
Interval 1	1483 ± 423.8	1575 ± 354.3	1441 ± 699.2	1098 ± 383.9
Interval 2	1109 ± 254.2	859.0 ± 473.1	595.8** ± 355.9 (−46%)	414.8** ± 304.0 (−63%)
Interval 3	781.1 ± 399.7	571.0 ± 444.6	338.4* ± 375.4 (−57%)	219.2** ± 197.1 (−72%)
Interval 4	614.5 ± 333.2	191.4** ± 257.3 (−69%)	118.8** ± 125.4 (−81%)	68.2** ± 76.9 (−89%)
Interval 5	366.5 ± 238.1	102.9* ± 133.3 (−72%)	86.7* ± 124.2 (−76%)	36.8** ± 57.9 (−90%)
Day 7, total	4855.1 ± 1067.8	4902.7 ± 1715.7	5543.0 ± 2268.9	5101.4 ± 1181.8
Day 14, total	5381.4 ± 1170.2	6496.6 ± 1988.8	6187.8 ± 2137.0	6758.9 ± 1291.9

From Kaspers et al. (2009b)
* $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)

Table 136. Mean terminal body weights and absolute and relative brain weights of rats administered fluxapyroxad once and observed for 14 days

	Dose (mg/kg bw)	Mean value ± SD							
		Males (*n* = 10/group)				Females (*n* = 10/group)			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
Terminal weight (g)	0	279.9 ± 8.1	—	—	—	185.4 ± 3.7	—	—	—
	125	281.8 ± 8.0	(+0.7)	—	—	190.8 ± 7.0	(+2.9)	—	—
	500	282.3 ± 14.6	(+0.9)	—	—	186.5 ± 5.2	(+0.6)	—	—
	2000	272.8 ± 12.7	(−2.5)	—	—	188.0 ± 4.9	(+1.4)	—	—
Brain (g)	0	1.952 ± 0.039	—	0.698 ± 0.029	—	1.844 ± 0.082	—	0.995 ± 0.038	—
	125	1.976 ± 0.023	(+1.2)	0.702 ± 0.017	(+0.5)	1.832 ± 0.088	(−0.7)	0.960 ± 0.036	(−3.5)
	500	1.946 ± 0.065	(−0.3)	0.691 ± 0.040	(−1.0)	1.868 ± 0.032	(+1.3)	1.002 ± 0.041	(+0.8)
	2000	1.944 ± 0.095	(−0.4)	0.713 ± 0.021	(+2.1)	1.804 ± 0.042	(−2.2)	0.960 ± 0.045	(−3.4)

From Kaspers et al. (2009b)
SD, standard deviation
[a] Compared with control. Values may not calculate exactly due to rounding.
[b] Percentage of body weight.

The NOAEL was 125 mg/kg bw in males and females, based on decreased motor activity (in both sexes) and decreased rearing (in males only) at 500 mg/kg bw (Kaspers et al., 2009b).

Table 137. Incidence of selected neurohistopathological lesions in rats administered fluxapyroxad once and observed for 14 days (perfusion-fixed animals)

	Incidence and severity[a] of finding							
	Males (n = 5/group)				Females (n = 5/group)			
	0 mg/kg bw	125 mg/kg bw	500 mg/kg bw	2000 mg/kg bw	0 mg/kg bw	125 mg/kg bw	500 mg/kg bw	2000 mg/kg bw
Tibial nerve, distal								
Axonal degeneration	0	0	0	1 [1.0]	0	0	0	0

From Kaspers et al. (2009b)

[a] [ ] mean severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked (Grade 4) and massive/severe (Grade 5). The mean severity is the sum of the gradings divided by the incidence.

In a 13-week neurotoxicity study, fluxapyroxad (purity 99.4%) was administered in the diet to groups of 10 male and 10 female Wistar Crl:WI(Han) rats at a concentration of 0, 200, 1000 or 5000 ppm (equal to doses of 0, 11.5, 57.7 and 302.2 mg/kg bw per day for males and 0, 13.4, 67.2 and 337.7 mg/kg bw per day for females, respectively) for 13 weeks. Feed consumption and body weights were recorded weekly, and a functional observational battery of tests, including a quantitative assessment of motor activity, was performed on all animals predosing and on study days 1, 22, 50 and 85. Clinical chemistry parameters were assessed at termination in five rats of each sex per dose. Five animals of each sex per dose were subjected to necropsy, postmortem examination, brain weight and dimensions recording, perfusion fixation and preservation of brain, dorsal root fibres and ganglia, ventral root fibres, spinal cord, eyes, optic, tibial and sciatic nerves, skeletal muscle and gross lesions. The remaining five animals of each sex per dose were subjected to gross pathological examination as well as histopathological evaluation of the liver.

Signs of systemic toxicity observed in this study were consistent with those observed in other repeated-dose studies in rats with fluxapyroxad. These consisted of tooth whitening in both sexes (Table 138) and slightly impaired body weight development in high-dose females (Table 139), changes in clinical chemistry parameters (increased serum GGT, total protein, albumin, globulin, cholesterol, triglyceride, urea, calcium, inorganic phosphate and magnesium levels, as well as decreased AST, glucose and bilirubin levels) (Table 140) at doses of 1000 ppm and above, and increased liver and thyroid weights (Table 141) at all doses tested, as well as enlarged livers (Table 142) and hypertrophy of hepatocytes (Table 143) at 1000 ppm and above.

Table 138. Clinical observations in rats administered fluxapyroxad for at least 91 days

	Incidence of finding							
	Males (n = 10/group)				Females (n = 10/group)			
	0 ppm	200 ppm	1000 ppm	5000 ppm	0 ppm	200 ppm	1000 ppm	5000 ppm
Tooth whitening, lower jaw	0	0	0	10	0	0	0	10
Alopecia, body or limbs	0	0	0	1	0	0	0	0
Faeces, discoloured dark	0	0	0	10	0	0	0	10

From Kaspers et al. (2009c)

Table 139. Mean body weight, body weight gain and cumulative feed consumption data for rats administered fluxapyroxad for at least 91 days

	Mean value ± SD							
	Males (n = 10/group)				Females (n = 10/group)			
	0 ppm	200 ppm	1000 ppm	5000 ppm	0 ppm	200 ppm	1000 ppm	5000 ppm
Body weight (g)								
- day 0	221.1 ± 4.7	223.2 ± 14.7	222.5 ± 9.9	228.8 ± 9.3	161.5 ± 5.5	163.1 ± 6.3	162.8 ± 7.6	169.7* ± 4.5 (+5.1%)
- day 91	415.3 ± 27.4	434.0 ± 41.9	427.5 ± 24.2	415.5 ± 28.0	254.7 ± 16.7	245.6 ± 12.8	250.2 ± 10.8	243.5 ± 13.1
% change[a]	—	*+4.5*	*+2.9*	*0.0*	—	*−3.6*	*−1.8*	*−4.4*
Overall body weight gain (g)[b]	194.2	210.8	205	186.7	93.3	82.5	87.4	73.8**
% change[a]	—	*+8.5*	*+5.6*	*−3.9*	—	*−11.5*	*−6.2*	*−20.9*
Cumulative feed consumption (g/animal)[c]								
- days 0–91[b]	1024	1871	1869	1933	1360	1343	1341	1358
% change[a]	—	*+2.6*	*+2.5*	*+6.0*	—	*−1.3*	*−1.4*	*−0.2*

From Kaspers et al. (2009c)

SD, standard deviation; * $P \leq 0.05$; ** $P < 0.01$

[a] Compared with controls. Values may not calculate exactly due to rounding of mean values (values given in the table are based on unrounded means).

[b] Standard deviations not provided in study report.

[c] Values calculated based on group mean daily feed consumption.

Table 140. Selected clinical chemistry findings in rats administered fluxapyroxad for at least 91 days

	Mean values ± SD							
	Males (n = 5/group)				Females (n = 5/group)			
	0 ppm	200 ppm	1000 ppm	5000 ppm	0 ppm	200 ppm	1000 ppm	5000 ppm
GGT (µkat/l)	0 ± 0	0 ± 0	0 ± 1	101** ± 23	2 ± 3	1 ± 3	0 ± 0	10 ± 10
AST (µkat/l)	2.11 ± 0.22	2.00 ± 0.37	2.32 ± 1.36	1.78 ± 0.23	1.47 ± 0.22	1.29 ± 0.15	1.22 ± 0.21	1.04** ± 0.18 (−29%)
Mg^{2+} (mmol/l)	0.90 ± 0.05	0.91 ± 0.04	0.91 ± 0.07	0.95 ± 0.08	0.93 ± 0.05	0.96 ± 0.06	1.01 ± 0.07	1.04* ± 0.06 (+12%)
ALT (µkat/l)	0.77 ± 0.16	0.74 ± 0.10	0.78 ± 0.20	0.84 ± 0.15	0.71 ± 0.13	0.60 ± 0.13	0.62 ± 0.08	0.52 ± 0.05
ALP (µkat/l)	1.26 ± 0.33	1.44 ± 0.38	1.34 ± 0.09	0.98 ± 0.09	0.58 ± 0.20	0.55 ± 0.23	0.47 ± 0.11	0.47 ± 0.07
Ca^{2+} (mmol/l)	2.55 ± 0.05	2.58 ± 0.04	2.64 ± 0.07	2.69** ± 0.04 (+5.5%)	2.62 ± 0.06	2.66 ± 0.07	2.69 ± 0.05	2.85** ± 0.06 (+8.8%)
PO_4^{3-}, inorganic (mmol/l)	1.76 ± 0.11	1.88 ± 0.09	2.06** ± 0.11 (+17%)	2.09** ± 0.12 (+19%)	1.45 ± 0.15	1.47 ± 0.20	1.58 ± 0.20	1.74 ± 0.17

	Mean values ± SD							
	Males (*n* = 5/group)				Females (*n* = 5/group)			
	0 ppm	200 ppm	1000 ppm	5000 ppm	0 ppm	200 ppm	1000 ppm	5000 ppm
Urea (mmol/l)	5.94 ± 0.70	5.86 ± 0.60	6.13 ± 0.60	6.34 ± 0.68	5.89 ± 0.50	6.26 ± 0.60	7.31** ± 0.16 (+24%)	7.57* ± 1.16 (+29%)
Glucose (mmol/l)	6.52 ± 0.37	5.64 ± 0.81	5.80 ± 0.70	3.99** ± 0.30 (−39%)	5.47 ± 0.20	5.52 ± 0.45	5.32 ± 0.54	4.85 ± 0.40
Bilirubin, total (µmol/l)	2.53 ± 0.39	2.09 ± 0.33	1.83 ± 0.39	2.04 ± 0.37	3.73 ± 0.47	3.00* ± 0.32 (−20%)	2.63* ± 0.46 (−29%)	2.37* ± 0.62 (−36%)
Protein, total (g/l)	64.36 ± 1.49	65.31 ± 0.93	67.38 ± 2.69	71.90** ± 2.18 (+12%)	67.21 ± 3.88	69.09 ± 3.10	71.56 ± 2.18	80.31** ± 3.05 (+19%)
Albumin (g/l)	37.75 ± 0.70	37.29 ± 0.53	38.16 ± 1.24	39.47* ± 0.86 (+4.6%)	40.96 ± 2.24	41.11 ± 1.11	42.01 ± 1.87	44.80* ± 1.23 (+9.4%)
Globulin (g/l)	26.61 ± 0.81	28.02* ± 0.82 (+5.3%)	29.22** ± 1.59 (+9.8%)	32.43** ± 1.49 (+22%)	26.25 ± 1.74	27.98 ± 2.11	29.55* ± 0.57 (+13%)	35.51** ± 2.06 (+35%)
Creatinine (µmol/l)	55.6 ± 3.7	55.1 ± 1.4	53.6 ± 2.6	54.5 ± 1.2	53.5 ± 1.3	55.3 ± 1.8	58.5* ± 3.0 (+9.3%)	58.7* ± 6.0 (+9.7%)
Triglycerides (mmol/l)	1.07 ± 0.32	1.07 ± 0.33	1.13 ± 0.22	1.37 ± 0.33	0.48 ± 0.11	0.48 ± 0.12	0.44 ± 0.10	1.11* ± 0.50 (+130%)
Cholesterol (mmol/l)	1.78 ± 0.55	2.01 ± 0.32	2.44 ± 0.41	3.54** ± 0.66 (+99%)	1.26 ± 0.09	1.52 ± 0.43	2.01** ± 0.29 (+59%)	3.88** ± 0.47 (+210%)

From Kaspers et al. (2009c)

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; SD, standard deviation; * $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)

Table 141. Terminal body and organ weights of rats administered fluxapyroxad for at least 91 days (non-perfusion-fixed animals)

	Dietary concentration (ppm)	Mean value ± SD							
		Males (*n* = 5/group)				Females (*n* = 5/group)			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
Terminal weight (g)	0	386.1 ± 18.5	—	—	—	233.4 ± 19.0	—	—	—
	200	411.9 ± 51.3	(+6.7)	—	—	222.6 ± 8.8	(−4.6)	—	—
	1000	411.1 ± 24.3	(+6.5)	—	—	232.4 ± 12.5	(−0.4)	—	—
	5000	401.4 ± 25.3	(+4.0)	—	—	222.4 ± 9.2	(−4.7)	—	—
Liver (g)	0	8.592 ± 0.569	—	2.224 ± 0.058	—	5.192 ± 0.585	—	2.225 ± 0.190	—

Table 141 (continued)

	Dietary concentration (ppm)	Mean value ± SD							
		Males (n = 5/group)				Females (n = 5/group)			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
	200	9.942 ± 1.164	(+15.7)	2.416** ± 0.055	(+8.6)	5.278 ± 0.283	(+1.7)	2.370 ± 0.059	(+6.5)
	1000	11.60** ± 0.42	(+35.1)	2.828** ± 0.141	(+27.2)	6.418** ± 0.43	(+23.6)	2.764** ± 0.178	(+24.3)
	5000	15.90** ± 1.78	(+85.0)	3.955** ± 0.303	(+77.8)	8.900** ± 0.919	(+71.4)	3.995** ± 0.279	(+79.6)
Thyroid gland (g)	0	0.019 ± 0.003	—	0.005 ± 0.001	—	0.013 ± 0.003	—	0.005 ± 0.001	—
	200	0.024* ± 0.002	(+24.7)	0.006 ± 0.001	(+17.7)	0.017* ± 0.002	(+31.3)	0.008* ± 0.001	(+38.5)
	1000	0.027* ± 0.004	(+39.2)	0.007 ± 0.001	(+27.2)	0.019* ± 0.002	(+50.0)	0.008** ± 0.001	(+52.5)
	5000	0.029** ± 0.003	(+49.5)	0.007** ± 0.0	(+43.0)	0.021** ± 0.002	(+60.9)	0.009** ± 0.001	(+71.1)

From Kaspers et al. (2009c)

SD, standard deviation; * $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)

[a] Compared with control. Values may not calculate exactly due to rounding.

[b] Percentage of body weight.

Table 142. Incidence of gross pathological findings in rats administered fluxapyroxad for at least 91 days (non-perfusion-fixed animals)

	Males (n = 5/group)				Females (n = 5/group)			
	0 ppm	200 ppm	1000 ppm	5000 ppm	0 ppm	200 ppm	1000 ppm	5000 ppm
Liver								
Enlarged	0	0	4	5	0	0	5	5
Focus	0	0	0	1	0	0	0	0
Prominent acinar pattern	0	0	0	1	0	0	0	0
Teeth								
Incisor, mandible, discoloration	0	0	0	4	0	0	0	5

From Kaspers et al. (2009c)

Treatment with fluxapyroxad did not result in any clinical (general clinical observations, functional observational battery and motor activity) or neurohistopathological indications of neurotoxicity.

The NOAEL for systemic effects could not be determined, based on the occurrence of increased absolute and relative thyroid weights in the absence of histopathological examination. The LOAEL for systemic effects was 200 ppm (equal to 11.5 mg/kg bw per day). It was concluded that fluxapyroxad did not elicit functional or morphological evidence of neurotoxicity at target dose levels up to 5000 ppm. Therefore, the NOAEL for neurotoxicity was 5000 ppm (equal to 302.2 mg/kg bw per day), the highest dose tested (Kaspers et al., 2009c).

Table 143. Incidence of selected histopathological lesions in the liver of rats administered fluxapyroxad for at least 91 days (non-perfusion-fixed animals)

	Incidence and severity[a] of finding							
	Males (*n* = 5/group)				Females (*n* = 5/group)			
	0 ppm	200 ppm	1000 ppm	5000 ppm	0 ppm	200 ppm	1000 ppm	5000 ppm
Liver								
Hepatocellular hypertrophy, central	0	5	5	5	0	0	5	5
		[1.2]	[2.0]	[3.8]			[3.0]	[4.0]
Lymphoid infiltration, (multi)focal	5	5	5	5	5	5	5	5
	[2.6]	[1.2]	[1.4]	[2.0]	[1.2]	[1.0]	[1.6]	[1.0]

From Kaspers et al. (2009c)

[a] [ ] mean severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked/severe (Grade 4) and massive/extreme (Grade 5). The mean severity is the sum of the gradings divided by the incidence.

(c) Immunotoxicity

In an immunotoxicity study, fluxapyroxad (purity 99.2%) was administered in the diet to C57BL/6J Rj mice (eight males per dose) at a concentration of 0, 500, 2000 or 6000 ppm (equal to doses of 0, 106, 450 and 1323 mg/kg bw per day) for 4 weeks. A similar group of mice, given daily oral (gavage) doses of cyclophosphamide at 12 mg/kg bw per day for 4 weeks, acted as a positive control group. All animals received a sensitizing intraperitoneal dose of sheep red blood cells (0.5 ml) on day 23, 5 days prior to termination. Body weights and feed and water consumption were measured weekly, and blood samples for total and differential white blood cell counts were withdrawn during week 4. All animals were subjected to necropsy after 4 weeks of treatment, and the weights of spleen and thymus were recorded. Lymphocyte counts and humoral immunity assessment (immunoglobulin M [IgM]) were performed on blood collected at study termination. Splenic tissue from all test, control and positive control animals was used as a source of splenocytes for assessment of the natural killer (NK) cell response.

All animals survived the scheduled treatment period, and there were no treatment-related clinical signs or effects on body weight gain or feed consumption at any dose level.

The parameters used for detection of potential test substance–related alterations in the morphology of the immune system included the determination of lymphoid organ weights (spleen and thymus) and the total lymphocyte count, as well as lymphocyte subpopulation distribution, including NK cells. Furthermore, functional parameters comprising the analysis of the primary humoral (IgM response) immune response to sheep red blood cells and NK cell activity were integrated in this study. None of the parameters mentioned above was affected by treatment with fluxapyroxad up to the highest dose level.

Concurrent treatment with the positive control substance, cyclophosphamide (12 mg/kg bw per day by oral gavage), induced clear signs of immunotoxicity, demonstrating the reliability of the test system under the study conditions employed.

The NOAEL for immunotoxic effects was 6000 ppm (equal to 1323 mg/kg bw per day), based on the lack of any morphological or functional change in the elements of the immune system investigated up to the limit dose. A LOAEL for immunotoxicity was not observed. The NOAEL for systemic effects was 6000 ppm (equal to 1323 mg/kg bw per day), the highest dose tested. A LOAEL for systemic effects was not observed (Kaspers et al., 2009d).

(d) Studies on metabolites

The acute, short-term and developmental toxicity and the genotoxic potential of the soil metabolites 5069089 (M700F001) and 5435595 (M700F002) and the plant metabolite 5570265 (M700F048), as well as the toxicokinetics of metabolites 5435595 and 5570265, were investigated. The studies were reported to comply with GLP and performed according to internationally accepted guidelines.

Metabolite 5069089 (M700F001)

In an acute oral toxicity study (acute toxic class method), single oral gavage doses of test substance Reg. No. 5069089 (purity 99.2%) suspended in olive oil were administered at 10 ml/kg bw to two groups of three female Crl:WI (Han) Wistar rats at a dose level of 2000 mg/kg bw.

No mortalities occurred. Clinical signs were observed in single animals and consisted of impaired general state, dyspnoea, piloerection and reduced defecation. All clinical signs resolved within 2 days. There were no macroscopic findings at necropsy in any animal. No overt body weight effects were evident. All animals gained weight during the study. Based on these results and under the conditions of this study, the acute oral LD_{50} was estimated to be greater than 2000 mg/kg bw (Cords & Lammer, 2009a).

In a short-term toxicity study, Reg. No. 5069089 (purity 99.2%) was administered in the diet to Wistar rats (10 of each sex per dose) at a target dose level of 0, 100, 300 or 1000 mg/kg bw per day for 13 weeks. Mean achieved doses were 0, 94.6, 285.7 and 953.6 mg/kg bw per day for males and 0, 98.8, 295.1 and 983.1 mg/kg bw per day for females, respectively. There were no deaths and no treatment-related adverse clinical signs, behavioural effects or effects on body weight gain, feed consumption or haematological, clinical chemistry or urine analysis parameters at any dose level. The significantly increased triglyceride value observed in the males at the high dose (Table 144) was regarded as test substance related, yet not adverse, as it is the only altered clinical pathology parameter. Furthermore, no treatment-related histopathological changes in the liver were observed. There were no effects on organ weights, gross pathology or histopathology.

Table 144. Serum triglyceride levels in rats administered M700F001 for at least 91 days

	Group mean value ± SD							
	Males (*n* = 10/group)				Females (*n* = 10/group)			
	0 mg/kg bw per day	100 mg/kg bw per day	300 mg/kg bw per day	1000 mg/kg bw per day	0 mg/kg bw per day	100 mg/kg bw per day	300 mg/kg bw per day	1000 mg/kg bw per day
Triglycerides (mmol/l)	0.92 ± 0.27	1.09 ± 0.31	1.10 ± 0.20	1.45** ± 0.41 (+58%)	0.62 ± 0.20	0.61 ± 0.25	0.72 ± 0.31	0.61 ± 0.15

From Kaspers et al. (2009e)
SD, standard deviation; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)

The NOAEL was 954 mg/kg bw per day, the highest dose tested. A LOAEL was not observed (Kaspers et al., 2009e).

In two independent microbial mutagenicity tests, *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* strain WP2*uvrA* were exposed to Reg. No. 5069089 (purity 99.2%) in dimethyl sulfoxide (DMSO) at five dose levels up to 5000 µg/plate, with or without exogenous metabolic activation (±S9). Triplicate plates were used per dose and per test condition. Vehicle and positive controls were included in each experiment. The plate incorporation

assay as well as the preincubation test were conducted. Concentrations of 20, 100, 500, 2500 and 5000 µg/plate were used in the plate incorporation assays with and without metabolic activation. For the preincubation assays, concentrations of 312.5, 625, 1250, 2500 and 5000 µg/plate were used. Because of high toxicity, strain TA1535 was additionally tested in the fourth experiment (preincubation assay) using 10, 50, 250, 1250 and 2500 µg/plate. In both the plate incorporation assay and the preincubation assay, a bacteriotoxic effect was observed, depending on the strain and test conditions, at concentrations of 2500 and 1250 µg/plate or above, respectively. Precipitation of the test substance did not occur up to the highest tested concentration.

The incidences of revertant colonies at all levels of Reg. No. 5069089, in all strains, both with and without S9, were comparable to the corresponding solvent control incidences. The efficacy of the S9 mix and the sensitivity of the test system to detect mutagenic agents were adequately demonstrated by the responses obtained with the non-activated and S9-activated positive controls.

Under the conditions of this study, Reg. No. 5069089 did not induce gene mutation in any of the strains employed, either with or without metabolic activation, at concentrations up to 5000 µg/plate (Schulz & Landsiedel, 2009a).

In an in vitro chromosomal aberration test, Chinese hamster lung fibroblasts (V79) were exposed to Reg. No. 5069089 (purity 99.2%), prepared in DMSO, to evaluate the clastogenic potential of test material at concentrations ranging from 250 to 2000 µg/ml (equivalent to 11.36 mmol/l) with or without S9. Dose levels for the main assay were determined following a preliminary cytotoxicity test. On the basis of these results, 2000 µg/ml was selected as the highest concentration for the short-term (4-hour) treatment assays in the presence or absence of S9 and continuous treatment 18-hour assay.

In the first and third experiments, the incidence of cells with structural aberrations and the incidence of polyploid cells in the groups treated with Reg. No. 5069089 were comparable to those of the negative control group in all three assays. In the second experiment, statistically significant increases were observed in the percentage of aberrant cells, including gaps, for the groups continuously treated with 250 and 1000 µg/ml and prepared after 18 hours. These increases were not reflected in the percentage of aberrant cells excluding gaps and were still within the historical control range and were thus considered as biologically irrelevant. Both positive controls induced structural chromosomal aberrations at a markedly higher incidence compared with the negative controls.

Reg. No. 5069089 did not induce chromosomal aberrations in cultured mammalian cells under the conditions of this study (Schulz & Landsiedel, 2009b).

In a mammalian cell forward gene mutation assay in Chinese hamster ovary (CHO) cells, Reg. No. 5069089 (purity 99.2%) was tested in vitro by assessing mutation of the *HPRT* locus. Two independent experiments were conducted in the presence and absence of metabolic activation. Based on the results of a preliminary cytotoxicity assay, concentrations of up to 2000 µg/ml (approximately 11.4 mmol/l) were used in the original and the confirmatory experiments. The treatment intervals in the absence of metabolic activation were 4 and 24 hours in the first and second experiments, respectively. The treatment interval for cultures treated with the test substance in the presence of metabolic activation was 4 hours for both experiments. Ethylmethanesulfonate (EMS) and methylcholanthrene (MCA) served as positive controls in the experiments without and with metabolic activation, respectively. In both experiments after the incubation period, treatment media were replaced by culture medium, and the cells were incubated for 6–8 days for expression of mutant cells. This was followed by incubation of cells in selection medium containing 6-thioguanine for about 1 week.

Cytotoxic effects, indicated by reduced cloning efficiencies of below 20% of the respective vehicle control, were not observed in any of the experiments, irrespective of treatment interval and the presence of metabolic activation. Relevant increases in the mutant frequency were not observed in the

original or the confirmatory studies. The positive control substances, however, induced a marked increase in mutant frequency. Based on the results of the study, it is concluded that under the conditions of the test, M700F01 does not induce forward mutations in mammalian cells in vitro (Schulz & Landsiedel, 2009c).

In an in vivo bone marrow micronucleus assay, Reg. No. 5069089 (purity 99.2%) prepared in suspensions in corn oil was administered via gavage to NMRI mice (five males per dose) once at a dose level of 0 (vehicle), 500, 1000 or 2000 mg/kg bw per day. The vehicle served as negative control, and cyclophosphamide and vincristine sulfate as positive controls. The animals were sacrificed 24 or 48 (additional high-dose and vehicle groups) hours after the administration, and the bone marrow of the two femora was prepared. After staining of the preparations, 2000 polychromatic erythrocytes were evaluated per animal and investigated for micronuclei. The normocytes with and without micronuclei occurring per 2000 polychromatic erythrocytes were also recorded.

There were no deaths, no treatment-related clinical signs and no apparent suppression of body weight gain at any dose level. The group mean frequencies of micronucleated polychromatic erythrocytes in the treated groups were similar to the vehicle control frequency, and none was statistically significantly different from the control frequency. There was no significant difference in the proportion of polychromatic erythrocytes relative to total erythrocytes between any of the Reg. No. 5069089–treated groups and the negative control group. The frequencies of micronucleated polychromatic erythrocytes and the proportion of polychromatic erythrocytes in both the vehicle control and positive control groups were within the range of historical control data for the performing laboratory. Both positive control chemicals (i.e. cyclophosphamide for clastogenic effects and vincristine sulfate for induction of spindle poison effects) led to the expected increase in the rate of polychromatic erythrocytes containing small (cyclophosphamide) or small and large (vincristine sulfate) micronuclei, thus demonstrating the sensitivity of the test system.

It was concluded that Reg. No. 5069089 did not induce the formation of micronucleated polychromatic erythrocytes in male mouse bone marrow cells under the conditions employed for this study at the maximum dose levels specified in the guidelines (Schulz & Landsiedel, 2009d).

In a developmental toxicity study in rabbits, Reg. No. 5069089 (purity 99.2%) was administered to mated female New Zealand White rabbits (31 per dose, 32 controls) via gavage, from day 6 to day 28 of gestation, at a dose level of 0, 40, 100 or 250 mg/kg bw per day at 10 ml/kg bw in aqueous carboxymethylcellulose (0.5% w/v). The animals were killed on day 29 after mating for reproductive assessment and fetal examination. Clinical signs, body weight and feed consumption were recorded. Adult females were examined macroscopically at necropsy on day 29 after mating, and all fetuses were examined macroscopically at maternal necropsy and subsequently by detailed internal visceral examination and skeletal examination.

There were no treatment-related maternal mortalities. No effects on maternal feed consumption or body weight development were observed. Likewise, no treatment-related necropsy findings were noted. Even though the conception rate of the time-mated rabbits was relatively low (72%, 87%, 90% and 84% at 0, 40, 100 and 250 mg/kg bw per day, respectively), a sufficient number of litters for evaluation was available (20, 25, 26 and 25 at 0, 40, 100 and 250 mg/kg bw per day, respectively). No treatment-related effects on gestational parameters (numbers of corpora lutea, implantation sites, preimplantation and post-implantation losses, early and late resorptions, dead and viable fetuses, placental or fetal weights or sex ratio) were observed.

Fetal examination did not reveal any treatment-related external, visceral or skeletal malformations, variations or unclassified observations.

A maternal LOAEL was not observed. The maternal NOAEL was 250 mg/kg bw per day, the highest dose tested. A developmental LOAEL was not observed. The developmental NOAEL was 250 mg/kg bw per day, the highest dose tested (Schneider et al., 2009b).

Metabolite 5435595 (M700F002)

The distribution of Reg. No. 5435595 in mouse blood cells, plasma and bone marrow after a single oral gavage administration was determined using a test substance formulation containing ^{14}C-labelled Reg. No. 5435595 (purity 98.9%) in corn oil. For this purpose, three male mice were treated orally with a nominal dose of 1000 mg/kg bw. The target quantity of radioactivity was about 2 MBq per animal. After 5 hours, the animals were sacrificed, and samples of the indicated tissues were analysed for test substance content. The analysis of the test substance formulation showed that animals received actual doses of 964.0, 966.6 and 445.5 mg/kg bw. As the achieved dose in the third animal was distinctly below the nominal value, its results were disregarded from the calculations. The mean radioactive dose of the two remaining animals was 1.96 MBq/animal. The mean total radioactive residues (TRR) in the bone marrow (22.03 µg Eq/g) after 5 hours were 0.002% of the applied dose, 0.006% in the plasma (14.84 µg Eq/g) and 0.004% in the blood cells (10.32 µg Eg/g) (Table 145).

Table 145. Radioactive residues in various tissues of male mice after a single treatment with ^{14}C-labelled M700F002

	No. 1	No. 2	No. 3	Mean[a]
Animal weight (g)	30.6	29.5	30.9	30.05
Specific activity (MBq/g)	—		—	67.56
Dose administered (mg/kg bw)	964.0	966.6	445.5	965.3
Radioactive dose (MBq/animal)	1.99	1.93	0.93	1.96
Blood cells (µg Eq/g) (% of administered dose)	8.29 (0.004%)	12.36 (0.003%)	8.63 (0.008%)	10.32 (0.004%)
Plasma (µg Eq/g) (% of administered dose)	12.50 (0.005%)	17.17 (0.007%)	13.43 (0.017%)	14.84 (0.006%)
Bone marrow (µg Eq/g) (% of administered dose)	5.27 (0.001%)	38.79 (0.003%)	11.77 0.005%)	22.03 (0.002%)

From Fabian & Landsiedel (2009b)
Eq, equivalent
[a] Mean value of animals 1 and 2.

Therefore, it is concluded that Reg. No. 5435595 is systemically biovailable, and its presence in the bone marrow and blood after an oral application is confirmed (Fabian & Landsiedel, 2009b).

In an acute oral toxicity study (up and down procedure), single oral gavage doses of Reg. No. 5435595 (purity 99%) were administered in olive oil, at 5 ml/kg bw, to two groups of three food-deprived female Wistar rats at a dose level of 2000 mg/kg bw. The animals were observed for 14 days post-treatment and then subjected to necropsy and postmortem examination.

No deaths occurred, and there were no macroscopic findings at necropsy in any animal. No overt body weight effects were evident. Clinical observation of the first test group revealed impaired general state, dyspnoea and piloerection in two animals from hour 2 through hour 5 after administration. No clinical signs or findings were observed in the second administration group. All animals gained weight during the study. Based on these results and under the conditions of this study, the acute oral LD_{50} was estimated to be greater than 2000 mg/kg bw (Cords & Lammer, 2009b).

In a short-term oral toxicity study in Wistar rats, four groups, each comprising five male and five female rats, were treated orally with Reg. No. 5435595 (purity 98.5%) at a dietary dose level of 0, 1500, 5000 or 15 000 ppm (equal to 0, 113.0, 375.9 and 1164.8 mg/kg bw per day for males and 0, 113.4, 394.8 and 1253.3 mg/kg bw per day for females, respectively) for 28 days. Clinical signs were

recorded daily, and a detailed examination of each animal was performed weekly (weeks 1–3). Feed and water consumption and body weights were recorded weekly, and blood samples for haematology and plasma chemistry and urine for urine analysis were taken after 4 weeks of treatment. All animals were sacrificed at the end of the observation period and subjected to necropsy and postmortem examination of major organs and tissues. Organ weights were recorded, and tissues from the control and high-dose groups and gross lesions from all animals were subjected to histopathological examination.

There were no deaths and no treatment-related clinical signs at any dose level during the general and detailed observations. Feed and water consumption and body weight gain were unaffected by treatment at all dose levels in both sexes. There were no treatment-related effects on the haematological or plasma chemistry profiles at any dose level in either sex. Urine analysis profiles were unaffected by treatment at all dose levels. At necropsy, there were no treatment-related macroscopic alterations or organ weight changes in either sex at any dose level and no treatment-related histopathological alterations at 1500 ppm.

The NOAEL was 15 000 ppm (equal to 1164.8 mg/kg bw per day for males and 1253.3 mg/kg bw per day for females), the highest dose tested. A LOAEL was not determined (Kaspers et al., 2008).

In a short-term oral toxicity study in Wistar rats, four groups, each comprising 10 male and 5 female rats, were treated orally with Reg. No. 5435595 (purity 98.5%) for 91 days in the diet at a target dose level of 0, 95.1, 285.3 or 958.4 mg/kg bw per day for males and 0, 98.0, 299.5 and 928.7 mg/kg bw per day for females, respectively. Clinical signs were recorded at least daily, a detailed physical examination was performed weekly and functional observational battery, grip strength and motor activity assessments were performed in week 13. Ophthalmoscopic examinations were performed predosing and in animals in the control and high-dose groups on day 91. Body weights and feed and water consumption were recorded weekly, and haematology, plasma clinical chemistry and urine analysis were performed in week 13 prior to necropsy. All animals, including decedents, were subjected to necropsy, postmortem examination, organ weight recording and tissue preservation. Major organs and tissues from the control and high-dose groups and liver, kidneys and gross lesions from all animals were processed and examined by light microscopy. Data were analysed statistically where appropriate.

There were no deaths and no treatment-related clinical signs at any dose level during the general and detailed observations. Feed and water consumption and body weight gain were unaffected by treatment at all dose levels in both sexes. There were no treatment-related effects on the haematological or plasma chemistry profiles at any dose level in either sex. Urine analysis profiles were unaffected by treatment at all dose levels. At necropsy, there were no treatment-related macroscopic alterations or organ weight changes in either sex at any dose level and no treatment-related histopathological alterations at 1500 ppm.

The NOAEL was 958.4 mg/kg bw per day in males and 928.7 mg/kg bw per day in females, the highest dose levels tested. A LOAEL was not determined (Kaspers et al., 2009f).

In two independent microbial mutagenicity tests, *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* strain WP2*uvrA* were exposed to Reg. No. 5435595 (purity 99.2%) in DMSO at five dose levels up to 5000 µg/plate, with or without exogenous metabolic activation (±S9). Triplicate plates were used per dose and per test condition. Vehicle and positive controls were included in each experiment. The plate incorporation assay as well as the preincubation test were conducted. Concentrations of 20, 100, 500, 2500 and 5000 µg/plate were used in the plate incorporation assays with and without metabolic activation. For the preincubation assays, concentrations of 312.5, 625, 1250, 2500 and 5000 µg/plate were used. Because of high toxicity, strain TA1535 was additionally tested in the fourth experiment (preincubation assay) using concentrations of 10, 50, 250, 1250 and 2500 µg/plate. In both the plate incorporation assay and the preincubation assay, a bacteriotoxic effect was observed, depending on the strain and test conditions,

at concentrations of 2500 and 1250 µg/plate or above, respectively. Precipitation of the test substance did not occur up to the highest tested concentration.

The incidences of revertant colonies at all levels of Reg. No. 5435595, in all strains, both with and without S9, were comparable to the corresponding solvent control incidences. The efficacy of the S9 mix and the sensitivity of the test system to detect mutagenic agents were adequately demonstrated by the responses obtained with the non-activated and S9-activated positive controls.

Under the conditions of this study, Reg. No. 5435595 did not induce gene mutation in any of the strains employed, either with or without metabolic activation, at concentrations up to 5000 µg/plate (Schulz & Landsiedel, 2007b).

In an in vitro chromosomal aberration test, Chinese hamster lung fibroblasts (V79) were exposed to Reg. No. 5435595 (purity 99.2%), prepared in DMSO, to evaluate the clastogenic potential of test material concentrations ranging from 250 to 2000 µg/ml (equivalent to 11.36 mmol/l) with or without S9. Dose levels for the main assay were determined following a preliminary cytotoxicity test. On the basis of these results, 2000 µg/ml was selected as the highest concentration for the short-term (4-hour) treatment assays in the presence or absence of S9 and continuous treatment 18-hour assay.

In the first experiment, the incidence of cells with structural aberrations and the incidence of polyploid cells in the groups treated with Reg. No. 5435595 were comparable with those of the negative control group in all three assays. In the second experiment, statistically significant increases were observed in the percentage of aberrant cells, including gaps, for the groups continuously treated with 250 and 1000 µg/ml and prepared after 18 hours. These increases were not reflected in the percentage of aberrant cells excluding gaps and were still within the historical control range and were thus considered as biologically irrelevant. Both positive controls induced structural chromosomal aberrations at a markedly higher incidence compared with the negative controls.

Reg. No. 54555 did not induce chromosomal aberrations in cultured mammalian cells under the conditions of this study (Schulz & Landsiedel, 2008d).

In a mammalian cell forward gene mutation assay in CHO cells, Reg. No. 5435595 (purity 99.2%) was tested in vitro by assessing mutation of the *HPRT* locus. Three independent experiments were conducted in the presence and absence of metabolic activation. Based on the results of a preliminary cytotoxicity assay, concentrations of up to 1650 µg/ml (approximately 10 mmol/l) were used in the original and the confirmatory experiments. The treatment intervals in the absence of metabolic activation were 4 and 24 hours in the first and second experiments, respectively. The treatment interval for cultures treated with the test substance in the presence of metabolic activation was 4 hours for both experiments. EMS and MCA served as positive controls in the experiments without and with metabolic activation, respectively. In both experiments, after the incubation period, treatment media were replaced by culture medium, and the cells were incubated for 6–8 days for expression of mutant cells. This was followed by incubation of cells in selection medium containing 6-thioguanine for about 1 week. In the second experiment, because of the induced cytotoxicity in the presence of metabolic activation at all tested concentrations, the experimental part had to be repeated in a third experiment using a different regimen of test substance concentrations (lowest dose 400 µg/ml instead of 500 µg/ml).

Relevant increases in the mutant frequency were not observed in the original or the confirmatory studies. The positive control substances, however, induced a marked increase in mutant frequency. Based on the results of the study, it is concluded that under the conditions of the test, Reg. No. 5435595 does not induce forward mutations in mammalian cells in vitro (Schulz & Landsiedel, 2008e).

In an in vivo bone marrow micronucleus assay, Reg. No. 5435595 (purity 99.3%) prepared in suspension in DMSO was administered via gavage to NMRI mice (five males per dose) once at a dose level of 0 (vehicle), 375, 750 or 1500 mg/kg bw per day. The vehicle served as negative control, and cyclophosphamide and vincristine sulfate as positive controls. The animals were sacrificed 24 or 48 (additional high-dose and vehicle groups) hours after the administration, and the bone marrow of the two femora was prepared. After staining of the preparations, 2000 polychromatic erythrocytes were evaluated per animal and investigated for micronuclei. The normocytes with and without micronuclei occurring per 2000 polychromatic erythrocytes were also recorded.

There were no deaths, no treatment-related clinical signs and no apparent suppression of body weight gain at any dose level. The bioavailability of the test substance after an oral application was confirmed in a separate study (Fabian & Landsiedel, 2009b). The group mean frequencies of micronucleated polychromatic erythrocytes in the treated groups were similar to the vehicle control frequency, and none was statistically significantly different from the control frequency. There was no significant difference in the proportion of polychromatic erythrocytes relative to total erythrocytes between any of the Reg. No. 5435595–treated groups and the negative control group. The frequencies of micronucleated polychromatic erythrocytes and the proportion of polychromatic erythrocytes in both the vehicle control and positive control groups were within the range of historical control data for the performing laboratory. Both positive control chemicals (i.e. cyclophosphamide for clastogenic effects and vincristine sulfate for induction of spindle poison effects) led to the expected increase in the rate of polychromatic erythrocytes containing small (cyclophosphamide) or small and large (vincristine sulfate) micronuclei, thus demonstrating the sensitivity of the test system.

It was concluded that Reg. No. 5435595 did not induce the formation of micronucleated polychromatic erythrocytes in male mouse bone marrow cells under the conditions employed for this study at the maximum dose levels specified in the guidelines (Schulz & Landsiedel, 2009a).

In a developmental toxicity study in rabbits, Reg. No. 5435595 (purity 99.3%) was administered to mated female New Zealand White rabbits (25 per dose, except for 35 in high-dose and 60 in control groups) via gavage, from day 6 to day 28 of gestation, at a dose level of 0, 100, 300 or 1000 mg/kg bw per day at 10 ml/kg bw in aqueous carboxymethylcellulose (0.5% w/v). The animals were killed on day 29 after mating for reproductive assessment and fetal examination. Clinical signs, body weight and feed consumption were recorded. Adult females were examined macroscopically at necropsy on day 29 after mating, and all fetuses were examined macroscopically at maternal necropsy and subsequently by detailed internal visceral examination and skeletal examination.

The high dose of 1000 mg/kg bw per day elicited signs of maternal toxicity, as indicated by clinical signs (Table 146), five abortions, five cases of mortality, decreased feed consumption (−12% during the treatment period), corroborated by an increased number of animals with reduced or no defecation, statistically significant decreases in body weight gain during gestation days 14–16 and 28–29 as well as a decrease in cumulative body weight gain by 21% during the entire study (Tables 147 and 148). Stomach erosions were noted in three high-dose dams. No treatment-related effects on gestational parameters (numbers of corpora lutea, implantation sites, preimplantation and post-implantation losses, early and late resorptions, dead and viable fetuses, placental or fetal weights or sex ratio) were observed.

Fetal examination did not reveal any treatment-related external, visceral or skeletal malformations, variations or unclassified observations.

The maternal NOAEL was 300 mg/bw kg per day, based on increased mortality, stomach erosions, abortions and decreased body weight gain at 1000 mg/kg bw per day. The developmental NOAEL was 300 mg/kg bw day, the highest dose tested, based on abortions at 1000 mg/kg bw per day (Schneider et al., 2009c).

Table 146. Clinical observations in rabbits administered M700F002 during days 6–28 of gestation

Dose (mg/kg bw per day)	Animal no.	Gestation day(s)	Observation
0 (control)	4	25	Abortion—sacrificed
	38	15	Blood in bedding
		15–16	Hypothermia, poor general state
		16	Abortion—sacrificed
	42	8–10	No defecation
	45	8–10	No defecation
100	60	21	Abortion—sacrificed
	66	9	Found dead
	71	26–27	Blood in bedding
300	91	9–20	No defecation
		20	Found dead
1000	107	17	Found dead
	108	25	Abortion—sacrificed
	110	17–19	Blood in bedding
		18–19	Poor general state, reduced defecation
		19	Lateral position, hypothermia, sacrificed moribund
	112	17	Poor general state, abortion—sacrificed
	117	21	Diarrhoea, lateral position, sacrificed moribund
	136	29	Abortion—sacrificed
	138	20	Abortion—sacrificed
	139	19–20	No defecation
		21	Found dead
	140	19–20	No defecation
		21	Found dead
	145	22–23	No defecation
		24	Poor general state, hypothermia, abortion—sacrificed

From Schneider et al. (2009c)

Table 147. Feed consumption and body weight development in rabbits administered M700F002 during days 6–28 of gestation

	Mean value ± SD (*n*)			
	0 mg/kg bw per day	100 mg/kg bw per day	300 mg/kg bw per day	1000 mg/kg bw per day
Feed consumption (g/animal per day)[a]				
Days 1–6	152.7 ± 42.44 (5)	148.2 ± 51.73 (5)	135.4 ± 50.37 (5)	144.6 ± 44.91 (5)
% change[b]	—	*−2.9*	*−11.3*	*−5.3*
Days 6–28	164.7 ± 22.70 (22)	158.2 ± 23.05 (22)	170.8 ± 23.65 (22)	144.3 ± 24.62 (22)
% change[b]	—	*−3.9*	*+3.7*	*−12.4*

Table 147 (continued)

	Mean value ± SD (*n*)			
	0 mg/kg bw per day	100 mg/kg bw per day	300 mg/kg bw per day	1000 mg/kg bw per day
Days 1–29	161.1 ± 27.25 (28)	155.2 ± 29.35 (28)	163.3 ± 31.98 (28)	143.3 ± 28.32 (28)
% change[b]	—	*−3.7*	*+1.3*	*−11.1*
Body weight gain (g)[c]				
Days 1–6	222 (43)	190.8 (19)	158.4 (17)	167.3 (27)
% change[b]	—	*−14.1*	*−28.6*	*−24.6*
Days 6–28	606.7 (41)	612.2 (17)	722.2 (17)	521.6 (19)
% change[b]	—	*+0.9*	*+19.0*	*−14.0*
Days 0–29	863	846.2	945.6	680.5**
% change[b]	—	*−1.9*	*+9.6*	*−21.1*

From Schneider et al. (2009c)
SD, standard deviation; ** $P \leq 0.01$ (Dunnett's test, two-sided)
[a] Mean of means.
[b] Compared with controls.
[c] Standard deviations not provided.

Table 148. Mean gravid uterus weights and net body weight change of pregnant rabbits administered M700F002 during days 6–28 of gestation

	Mean value ± SD			
	0 mg/kg bw per day	100 mg/kg bw per day	300 mg/kg bw per day	1000 mg/kg bw per day
Gravid uterus weight (g)	474.8 ± 114.07	466.2 ± 115.76	488.9 ± 149.29	404.4 ± 114.94
Carcass weight (g)	3275.7 ± 253.12	3197.5 ± 309.67	3283.1 ± 186.57	3201.6 ± 333.17
Net weight change from day 6 (g)	168.6 ± 240.92	181.2 ± 195.78	298.3 ± 209.84	132.7 ± 302.12

From Schneider et al. (2009c)
SD, standard deviation

Metabolite Reg. No. 5570265 (M700F048)

The distribution of Reg. No. 5570265 in mouse blood cells, plasma bone marrow and liver after a single oral gavage administration was determined using a test substance formulation containing ^{14}C-labelled Reg. No. 5570265 (purity 98.3%) in corn oil. For this purpose, three male mice were treated orally with a nominal dose of 1000 mg/kg bw. The target quantity of radioactivity was about 2 MBq/animal. After 5 hours, the animals were sacrificed, and samples of the indicated tissues were analysed for test substance content. The analysis of the test substance formulation showed that the animals received a mean actual dose of 841.1 mg/kg bw (mean radioactive dose 1.9 MBq/animal). The mean TRR in the bone marrow (1.61 µg Eq/g) were 0.0002% of the applied dose. In the liver (32.82 µg Eq/g), 0.230% of the applied dose was found after 5 hours. The mean TRR in the blood cells (0.59 µg Eq/g) and plasma (1.51 µg Eq/g) were 0.0005% and 0.0011%, respectively (Table 149).

Therefore, it is concluded that Reg. No. 5570265 is systemically biovailable, and its presence in the bone marrow and blood after an oral application is confirmed (Fabian & Landsiedel, 2009c).

Table 149. Radioactive residues in various tissues of male mice after a single treatment with ^{14}C-labelled M700F048

	No. 1	No. 2	No. 3	Mean	SD
Animal weight (g)	29.1	31.1	31.3	30.5	1.22
Specific activity (MBq/g)	—	—	—	74.12	—
Dose administered (mg/kg bw)	861.3	833.7	828.4	841.1	17.7
Radioactive dose (MBq/animal)	1.86	1.92	1.92	1.90	0.03
Blood cells (µg Eq/g) (% of administered dose)	0.34 (0.0003%)	1.05 (0.0010%)	0.39 (0.0002%)	0.59 (0.0005%)	0.40 (0.0004%)
Plasma (µg Eq/g) (% of administered dose)	0.86 (0.0007%)	2.95 (0.0022%)	0.72 (0.0002%)	1.51 (0.0011%)	1.25 (0.0010%)
Bone marrow (µg Eq/g) (% of administered dose)	1.03 (0.0001%)	1.63 (0.0002%)	2.18 (0.0002%)	1.61 (0.0002%)	0.58 (0.0000%)
Liver (µg Eq/g) (% of administered dose)	16.31 (0.113%)	67.19 (0.456%)	14.97 (0.121%)	32.82 (0.230%)	29.77 (0.196%)

From Fabian & Landsiedel (2009c)
Eq, equivalent; SD, standard deviation

In an acute oral toxicity study (acute toxic class method), a single oral gavage dose of Reg. No. 5570265 (purity 93.7%) at 2000 mg/kg bw was administered in olive oil, at 5 ml/kg bw, to two groups of three feed-deprived female Wistar rats. All surviving animals were observed for 14 days post-treatment and then subjected to necropsy and postmortem examination.

No mortality occurred during the study. Clinical observation revealed impaired general state, dyspnoea, piloerection and salivation from hour 0 up to study day 3 after administration in one animal of the first test group. In the second test group, two animals showed diarrhoea at hour 3 after administration, whereas the third animal revealed impaired general state, piloerection, exsiccosis and reduced faeces in combination with diarrhoea from study day 2 up to study day 3 after administration. There were no macroscopic findings at necropsy in any animal. No overt body weight effects were evident, and all animals gained weight during the study. Based on these results and under the conditions of this study, the acute oral LD_{50} was estimated to be greater than 2000 mg/kg bw (Cords & Lammer, 2009c).

In a short-term oral toxicity study in Wistar rats, four groups, each comprising 10 male and 5 female rats, were treated orally with Reg. No. 5570265 (purity 93.7%) in the diet at a target dose level of 0, 50, 200 or 1000 mg/kg bw per day for 28 days. Dietary concentrations of the test substance were adjusted weekly based on actual body weight and feed consumption to meet the intended nominal dose levels. The calculated mean daily intakes for males were 47.1 and 189.3 mg/kg bw per day in the low- and mid-dose animals. Intake could not be calculated for high-dose males due to food spillage. The calculated mean daily intakes for females were 0, 51.4, 208.2 and 1477.8 mg/kg bw per day. Clinical signs were recorded daily, a detailed examination of each animal was performed weekly (weeks 1–3) and functional observational battery, grip strength and motor activity assessments were performed in week 4. Ophthalmoscopic examinations were performed predosing and in animals in the control and high-dose groups on day 27. Feed consumption and body weights were recorded weekly, and blood samples for haematology and plasma chemistry and urine for urine analysis were taken after 4 weeks of treatment. All animals were sacrificed at the end of the observation period and subjected to necropsy and postmortem examination of major organs and tissues. Organ weights were recorded, and tissues from the control and high-dose groups and gross lesions from all animals were subjected to histopathological examination.

There were no deaths and no treatment-related clinical signs at any dose level during the general and detailed observations. Decreased body weights and impaired body weight development were observed in both sexes at the target dose level of 1000 mg/kg bw per day (Table 150). Feed consumption was increased in females at the high dose, but could not be determined in males due to spillage (Table 151). Changes in the liver were decreased bile acid and bilirubin levels (Table 152), slightly increased liver weights (Table 153) as well as a marginal centrilobular hepatocellular hypertrophy. These effects were considered to represent an adaptive response in the liver. In the blood, slightly decreased absolute and relative monocyte counts were observed at nominal doses of 200 mg/kg bw per day and above in males only (Table 154). This effect was not considered adverse in the absence of a dose–response relationship. Urine analysis profiles were unaffected by treatment at all dose levels.

Table 150. Mean body weight and body weight gain of rats administered M700F048 for 28 days

	Mean value ± SD							
	Males (n = 10/group)				Females (n = 10/group)			
	0 mg/kg bw per day	50 mg/kg bw per day	200 mg/kg bw per day	1000 mg/kg bw per day	0 mg/kg bw per day	50 mg/kg bw per day	200 mg/kg bw per day	1000 mg/kg bw per day
Body weight (g)								
- day 0	175.9 ± 6.9	178.2 ± 6.2	176.8 ± 5.8	176.4 ± 5.1	139.5 ± 6.0	138.1 ± 7.3	135.9 ± 5.9	139.6 ± 6.4
- day 28	285.4 ± 14.6	293.5 ± 19.9	281.1 ± 13.6	262.0** ± 11.7	188.0 ± 14.5	187.5 ± 10.7	180.9 ± 10.8	170.3** ± 9.1
% change[a]	—	*+2.8*	*−1.5*	*−8.2*	—	*−0.3*	*−3.8*	*−9.4*
Overall body weight gain (g)	109.5 ± 15.2	115.3 ± 15.2	104.3 ± 11.6	85.6** ± 11.7	48.6 ± 11.1	49.4 ± 9.9	45.0 ± 5.7	30.6** ± 7.5
% change[a]	—	*+5.3*	*−4.8*	*−21.9*	—	*+1.7*	*−7.4*	*−36.9*

From Kaspers et al. (2009g)
SD, standard deviation; * $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)
[a] Compared with controls.

Table 151. Cumulative feed consumption of rats administered M700F048 for 28 days

	Mean value							
	Males (n = 10/group)				Females (n = 10/group)			
	0 mg/kg bw per day	50 mg/kg bw per day	200 mg/kg bw per day	1000 mg/kg bw per day	0 mg/kg bw per day	50 mg/kg bw per day	200 mg/kg bw per day	1000 mg/kg bw per day
Cumulative feed consumption (g/animal)								
Days 0–28[a]	589	597	596	NC	443	438	422	610
% change[b]	—	*+1.4*	*+1.3*	*NC*	—	*−1.3*	*−4.7*	*+37.8*

From Kaspers et al. (2009g)
NC, not calculable due to food spillage
[a] Standard deviations not provided in study report.
[b] Compared with controls. Values were calculated based on mean individual daily feed consumption. Values may not calculate exactly due to rounding of mean values.

Table 152. Selected clinical chemistry findings in rats administered M700F048 for 28 days

	Group mean value ± SD							
	Males (n = 10/group)				Females (n = 10/group)			
	0 mg/kg bw per day	50 mg/kg bw per day	200 mg/kg bw per day	1000 mg/kg bw per day	0 mg/kg bw per day	50 mg/kg bw per day	200 mg/kg bw per day	1000 mg/kg bw per day
Bilirubin (µmol/l)	2.51 ± 0.32	2.27 ± 0.27	2.08* ± 0.36 (−17%)	1.99** ± 0.21 (−21%)	2.23 ± 0.60	2.17 ± 0.44	1.55** ± 0.27 (−30%)	1.50** ± 0.34 (−33%)
Bile acid (µmol/l)	24.0 ± 12	23.0 ± 13	18.0 ± 6	8.0** ± 5 (−67%)	39.0 ± 14	27.0* ± 10 (−31%)	26.0 ± 14	15.0** ± 8 (−62%)
Cl^- (mmol/l)	102.2 ± 1.5	102.0 ± 1.7	102.5 ± 2.1	102.6 ± 1.4	102.2 ± 1.2	101.7 ± 1.0	102.3 ± 1.6	101.0* ± 0.9 (−1.2%)
Triglycerides (mmol/l)	0.97 ± 0.34	0.69 ± 0.23	0.74 ± 0.21	0.87 ± 0.57	0.33 ± 0.08	0.32 ± 0.09	0.41 ± 0.12	0.70** ± 0.26 (+110%)

From Kaspers et al. (2009g)

SD, standard deviation; * $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)

Table 153. Selected mean absolute and relative organ weights of rats administered M700F048 for 28 days

	Dose (mg/kg bw per day)	Mean value ± SD							
		Males (n = 10/group)				Females (n = 10/group)			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
Terminal weight (g)	0	263.6 ± 13.7	—	—	—	177.7 ± 11.6	—	—	—
	50	270.4 ± 19.7	(+2.6)	—	—	176.5 ± 8.9	(−0.7)	—	—
	200	259.8 ± 12.8	(−1.4)	—	—	174.1 ± 10.1	(−2.0)	—	—
	2000	242.4** ± 9.8	(−8.0)	—	—	161.5** ± 7.8	(−9.1)	—	—
Adrenal gland (mg)	0	53.9 ± 5.8	—	0.02	—	68.4 ± 4.2	—	0.039	—
	50	57.6 ± 4.6	(+6.9)	0.021	(+4.6)	70.8	(+3.5)	0.040	(+3.8)
	200	55.9 ± 6.3	(+3.7)	0.021	(+5.1)	73.2	(+7.0)	0.042	(+8.7)
	2000	59.9 ± 8.0	(+11.1)	0.025**	(+20.8)	64.3	(−6.0)	0.040	(+3.3)
Brain (g)	0	1.883 ± 0.086	—	0.716	—	1.804	—	1.018	—
	50	1.935 ± 0.081	(+2.8)	0.719	(+0.4)	1.850	(+2.5)	1.050	(+3.1)
	200	1.901 ± 0.079	(+1.0)	0.733	(+2.4)	1.834	(+1.7)	1.054	(+3.5)
	2000	1.893 ± 0.039	(+0.5)	0.782**	(+9.2)	1.788	(−0.9)	1.109**	(+8.9)

Table 153 (continued)

	Dose (mg/kg bw per day)	Mean value ± SD							
		Males (*n* = 10/group)				Females (*n* = 10/group)			
		Absolute weight	% change[a]	Relative weight[b]	% change[a]	Absolute weight	% change[a]	Relative weight[b]	% change[a]
Heart (g)	0	0.818 ± 0.055	—	0.31	—	0.647	—	0.364	—
	50	0.842 ± 0.08	(+2.9)	0.312	(+0.6)	0.627	(−3.1)	0.356	(−2.2)
	200	0.791 ± 0.047	(−3.3)	0.305	(−1.6)	0.621	(−4.0)	0.356	(−2.2)
	2000	0.741** ± 0.032	(−9.4)	0.306	(−1.3)	0.587	(−9.3)	0.363	(−0.3)
Kidney (g)	0	1.792 ± 0.149	—	0.679	—	1.351	—	0.762	—
	50	1.871 ± 0.181	(+4.4)	0.693	(+2.1)	1.368	(+1.3)	0.775	(+1.7)
	200	1.729 ± 0.094	(−3.5)	0.667	(−1.8)	1.344	(−0.5)	0.770	(+1.0)
	2000	1.777 ± 0.107	(−0.8)	0.733**	(+8.0)	1.242	(−8.1)	0.770	(+1.0)
Liver (g)	0	6.603 ± 0.566	—	2.502	—	4.756	—	2.678	—
	50	6.706 ± 0.716	(+1.6)	2.477	(−1.0)	4.642	(−2.4)	2.629	(−1.8)
	200	6.490 ± 0.477	(−1.7)	2.497	(−0.2)	4.701	(−1.2)	2.699	(+0.8)
	2000	7.152* ± 0.37	(+8.3)	2.95**	(+17.9)	5.248**	(+10.3)	3.258**	(+21.7)
Ovary (mg)	0	—	—	—	—	93.5	—	0.053	—
	50	—	—	—	—	88.7	(−5.1)	0.050	(−5.7)
	200	—	—	—	—	84.8	(−9.3)	0.049	(−7.5)
	2000	—	—	—	—	73.2**	(−21.7)	0.045	(−15.1)
Testis (g)	0	2.969 ± 0.240	—	1.13	—	—	—	—	—
	50	3.023 ± 0.376	(+1.8)	1.119	(−1.0)	—	—	—	—
	200	3.025 ± 0.209	(+1.9)	1.167	(+3.3)	—	—	—	—
	2000	3.041 ± 0.282	(+2.4)	1.256**	(+11.2)	—	—	—	—
Thymus (mg)	0	413.7 ± 95.7	—	0.156	—	415.4	—	0.233	—
	50	424.8 ± 59.2	(+2.7)	0.157	(+0.6)	396.8	(−4.5)	0.224	(−3.9)
	200	426.8 ± 73.2	(+3.2)	0.164	(+5.1)	404.9	(−2.5)	0.232	(−0.4)
	2000	358.0 ± 86.6	(−13.5)	0.147	(−5.8)	332.0**	(−20.1)	0.205	(−12.0)

From Kaspers et al. (2009g)
SD, standard deviation; * $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)
[a] Compared with control. Values may not calculate exactly due to rounding.
[b] Percentage of body weight.

Table 154. Selected haematological findings in rats administered M700F048 for 28 days

	Group mean value ± SD							
	Males (*n* = 10/group)				Females (*n* = 10/group)			
	0 mg/kg bw per day	50 mg/kg bw per day	200 mg/kg bw per day	1000 mg/kg bw per day	0 mg/kg bw per day	50 mg/kg bw per day	200 mg/kg bw per day	1000 mg/kg bw per day
Haemoglobin (mmol/l)	9.0 ± 0.4	9.0 ± 0.4	9.3* ± 0.2 (+3.3%)	9.3* ± 0.2 (+3.3%)	9.0 ± 0.4	8.9 ± 0.2	8.8 ± 0.3	8.7 ± 0.4
MCV (fl)	50.1 ± 1.2	51.5* ± 1.3 (+2.8%)	52.0** ± 0.7 (+3.8%)	51.7** ± 0.8 (+3.2%)	51.3 ± 1.2	51.6 ± 1.6	51.4 ± 1.0	51.1 ± 1.0
MCH (fmol)	1.09 ± 0.03	1.13* ± 0.05 (+3.7%)	1.14** ± 0.04 (+4.6%)	1.13** ± 0.01 (+3.7%)	1.14 ± 0.03	1.16 ± 0.06	1.16 ± 0.04	1.15 ± 0.03
White blood cells (10^9/l)	6.09 ± 2.04	5.63 ± 0.98	5.95 ± 2.04	5.08 ± 1.35	4.43 ± 1.08	4.06 ± 1.52	4.40 ± 1.31	4.42 ± 1.11
Monocytes (%)	2.0 ± 0.7	1.5 ± 0.3	1.3** ± 0.3 (−35%)	1.3* ± 0.4 (−35%)	1.4 ± 0.4	1.5 ± 0.6	1.2 ± 0.3	1.3 ± 0.9
Monocytes (10^{12}/l)	0.12 ± 0.07	0.08 ± 0.071	0.07* ± 0.02 (−42%)	0.06** ± 0.02 (−42%)	0.06 ± 0.02	0.06 ± 0.03	0.05 ± 0.02	0.06 ± 0.06
Prothrombin time (HQT, s)	37.2 ± 2.4	37.7 ± 1.5	37.4 ± 2.3	38.6 ± 1.7	35.4 ± 1.6	34.4 ± 2.1	35.5 ± 1.2	32.6* ± 2.3 (−7.9%)

From Kaspers et al. (2009g)
HQT, Hepato-Quick test; MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume; * $P \leq 0.05$; ** $P \leq 0.01$ (Kruskal-Wallis and Wilcoxon test, two-sided)

The NOAEL was 200 mg/kg bw per day (equal to 189.3 mg/kg bw per day in males and 208.2 mg/kg bw per day in females), based on decreased body weights and feed consumption at 1000 mg/kg bw per day (target dose) in males and 1478 mg/kg bw per day in females (Kaspers et al., 2009g).

In two independent microbial mutagenicity tests, *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* strain WP2*uvrA* were exposed to Reg. No. 5570265 (purity 93.7%) in DMSO at five concentrations up to 5500 µg/plate, with or without exogenous metabolic activation (±S9). Triplicate plates were used per dose and per test condition. Vehicle and positive controls were included in each experiment. The plate incorporation assay as well as the preincubation test were conducted. Concentrations of 22, 110, 550, 2750 and 5500 µg/plate were used in the plate incorporation assays with and without metabolic activation. For the preincubation assays, concentrations of 22, 110, 550, 2750 and 5500 µg/plate were used. In both the plate incorporation assay and the preincubation assay, a weak bacteriotoxic effect was observed in strain TA1537 at concentrations of 2750 µg/plate and above and 5500 µg/plate, respectively. Precipitation of the test substance did not occur up to the highest tested concentration.

The incidences of revertant colonies at all concentrations of Reg. No. 5570265, in all strains, both with and without S9, were comparable to the corresponding solvent control incidences. The

efficacy of the S9 mix and the sensitivity of the test system to detect mutagenic agents were adequately demonstrated by the responses obtained with the non-activated and S9-activated positive controls.

Under the conditions of this study, Reg. No. 5570265 did not induce gene mutation in any of the strains employed, either with or without metabolic activation, at concentrations up to 5500 µg/plate (Schulz & Landsiedel, 2009f).

In an in vitro chromosomal aberration test, Chinese hamster lung fibroblasts (V79) were exposed to Reg. No. 5570265 (purity 96.1%), prepared in DMSO, to evaluate the clastogenic potential of test material concentrations ranging from 62.5 to 1500 µg/ml with or without S9. Concentrations for the main assay were determined following a preliminary cytotoxicity test. On the basis of these results, 1500 µg/ml was selected as the highest concentration for the short-term (4-hour) treatment assays in the presence or absence of S9 and continuous treatment 18-hour assay. The highest concentrations scored in cultures without metabolic activation were 750 µg/ml in pulse-treated cultures (first experiment) and 375 and 500 µg/ml in continuously treated cultures prepared after 18 and 28 hours, respectively (second experiment). In the presence of metabolic activation, cultures treated with 1000 µg/ml (first and second experiments) and 1200 µg/ml (third experiment) were the highest scorable cultures. In all cases, cultures treated with a higher concentration either were not scorable or showed overt cytotoxicity.

A relevant increase in the number of aberrant cells was not observed at any of the tested concentrations without metabolic activation in any of the performed experiments (Tables 155, 156, 157, 158, 159 and 160). However, in the presence of metabolic activation, a statistically significant and reproducible increase in the percentage of aberrant cells was induced at concentrations of 1000 and 1200 µg/ml and a preparation interval of 28 hours. Both positive controls induced structural chromosomal aberrations at a markedly higher incidence compared with the negative controls.

Based on the results of this study, M700F048 is considered to have a clastogenic potential in vitro in Chinese hamster V79 cells in the presence of metabolic activation (Schulz & Landsiedel, 2009g).

In a mammalian cell forward gene mutation assay in CHO cells, Reg. No. 5570265 (purity 93.7%) was tested in vitro by assessing mutation of the *HPRT* locus. Two independent experiments were conducted in the presence and absence of metabolic activation. Based on the results of a preliminary cytotoxicity assay, concentrations of 62.5–1500 µg/ml were used in the original experiment using a 4-hour exposure period with and without metabolic activation. In the second experiment, concentrations of 15.6–750 µg/ml were used without metabolic activation using a 24-hour exposure and 125–1500 µg/ml with metabolic activation using a 4-hour exposure. EMS and MCA served as positive controls in the experiments without and with metabolic activation, respectively. In both experiments, after the incubation period, treatment media were replaced by culture medium, and the cells were incubated for 7–9 days for expression of mutant cells. This was followed by incubation of cells in selection medium containing 6-thioguanine for about 1 week.

Cytotoxic effects indicated by reduced cloning efficiencies of below 20% of the respective vehicle control were observed in both experiments with or without metabolic activation. Relevant increases in the mutant frequency were not observed in the original or the confirmatory studies. The positive control substances, however, induced a marked increase in mutant frequency. Based on the results of the study, it is concluded that under the conditions of the test, Reg. No. 5570265 does not induce forward mutations in mammalian cells in vitro (Schulz & Landsiedel, 2009h).

Table 155. Chromosomal aberration test with M700F048 without metabolic activation (4-hour treatment, harvest after 18 hours): Experiment 1

	Culture	No. of metaphases	Mitotic index	Aberrant cells including gaps		Aberrant cells excluding gaps		Exchange figures		Multiple aberrations		Chromosome disintegrations		Aneuploidy		Polyploidy		Endopolyploidy	
		n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Vehicle DMSO	A	100	8.0	6	6.0	3	3.0	1	1.0	0	0.0	0	0.0	0	0.0	3	2.9	0	0.0
	B	100	9.8	1	1.0	1	1.0	1	1.0	0	0.0	0	0.0	0	0	0	0.0	0	0.0
	A + B	200	8.9	7	3.5	4	2.0	2	1.0	0	0.0	0	0.0	0	0.0	3	1.5	0	0.0
M700F048																			
375 µg/ml	A	100	6.5	8	8.0	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	7.1	3	3.0	2	2.0	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	200	6.8	11	5.5	4	2.0	2	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
500 µg/ml	A	100	8.0	5	5.0	3	3.0	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	8.1	3	3.0	3	3.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	200	8.1	8	4.0	6	3.0	3	1.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
750 µg/ml	A	100	7.6	10	10.0	4	4.0	3	3.0	0	0.0	0	0.0	0	0.0	2	2.0	0	0.0
	B	100	6.1	5	5.0	4	4.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	200	6.9	15	7.5	8	4.0	4	2.0	0	0.0	0	0.0	0	0.0	2	1.0	0	0.0
Positive control EMS																			
500 µg/ml	A	50	6.0	11	22.0	9	18.0	6	12.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	50	10.4	7	14.0	7	14.0	3	6.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	100	8.2	18	18.0**	16	16.0**	9	9.0**	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

From Schulz & Landsiedel (2009g)

DMSO, dimethyl sulfoxide; EMS, ethylmethanesulfonate; * $P \leq 0.05$; ** $P \leq 0.01$ (Fisher’s exact test, one-sided, with Bonferoni-Holm corrections)

Table 156. Chromosomal aberration test with M700F048 with metabolic activation (4-hour treatment, harvest after 18 hours): Experiment 1

	Culture	No. of metaphases	Mitotic index	Aberrant cells including gaps		Aberrant cells excluding gaps		Exchange figures		Multiple aberrations		Chromosome disintegrations		Aneuploidy		Polyploidy		Endopolyploidy	
		n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Vehicle DMSO	A	100	16.5	6	6.0	3	3.0	2	2.0	0	0.0	0	0.0	0	0.0	1	1.0	3	2.9
	B	100	13.7	1	1.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	1	1.0	4	3.8
	A + B	200	15.1	7	3.5	4	2.0	2	1.0	0	0.0	0	0.0	0	0.0	2	1.0	7	3.3
M700F048																			
500 µg/ml	A	100	9.8	5	5.0	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	3	2.9	0	0.0
	B	100	10.3	1	1.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	200	10.1	6	3.0	3	1.5	0	0.0	0	0.0	0	0.0	0	0.0	3	1.5	0	0.0
750 µg/ml	A	100	8.9	4	4.0	3	3.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	9.9	1	1.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	200	9.4	5	2.5	4	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
1000 µg/ml	A	100	8.2	7	7.0	3	3.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	9.6	2	2.0	1	1.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	200	8.9	9	4.5	4	2.0	2	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Positive control CP																			
0.5 µg/ml	A	50	6.2	16	32.0	14	28.0	7	14.0	1	2.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	50	7.4	8	16.0	8	16.0	4	8.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	100	6.8	24	24.0**	22	22.0**	11	11.0**	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0

From Schulz & Landsiedel (2009g)

CP, cyclophosphamide; DMSO, dimethyl sulfoxide; ** $P \leq 0.01$ (Fisher's exact test, one-sided, with Bonferoni-Holm corrections)

Table 157. Chromosomal aberration test with M700F048 without metabolic activation (18-hour treatment, harvest after 18 hours): Experiment 2

	Culture	No. of metaphases	Mitotic index	Aberrant cells including gaps		Aberrant cells excluding gaps		Exchange figures		Multiple aberrations		Chromosome disintegrations		Aneuploidy		Polyploidy		Endopolyploidy	
		n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Vehicle DMSO	A	100	8.2	3	3.0	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	6.2	5	5.0	2	2.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	200	7.2	8	4.0	4	2.0	1	0.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
M700F048																			
125 µg/ml	A	100	7.8	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	6.9	5	5.0	5	5.0	4	4.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	200	7.4	7	3.5	5	2.5	4	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
250 µg/ml	A	100	6.9	1	1.0	1	1.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	6.3	3	3.0	3	3.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	200	6.6	4	2.0	4	2.0	2	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
375 µg/ml	A	100	9.7	1	1.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	6.6	2	2.0	1	1.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	200	8.2	3	1.5	2	1.0	1	0.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Positive control EMS																			
500 µg/ml	A	50	8.7	11	22.0	11	22.0	9	18.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	50	8.8	10	20.0	9	18.0	5	10.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	100	8.8	21	21.0**	20	20.0**	14	14.0**	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

From Schulz & Landsiedel (2009g)

DMSO, dimethyl sulfoxide; EMS, ethylmethanesulfonate; ** $P \leq 0.01$ (Fisher's exact test, one-sided, with Bonferoni-Holm corrections)

Table 158. Chromosomal aberration test with M700F048 without metabolic activation (18-hour treatment, harvest after 28 hours): Experiment 2

	Culture	No. of metaphases	Mitotic index	Aberrant cells including gaps		Aberrant cells excluding gaps		Exchange figures		Multiple aberrations		Chromosome disintegrations		Aneuploidy		Polyploidy		Endopolyploidy	
		n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Vehicle DMSO	A	100	12.7	4	4.0	2	2.0	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	10.7	9	9.0	5	5.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	200	11.7	13	6.5	7	3.5	3	1.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
M700F048																			
500 μg/ml	A	100	7.0	4	4.0	3	3.0	2	2.0	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0
	B	100	6.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	200	6.5	5	2.5	3	1.5	2	1.0	0	0.0	0	0.0	0	0.0	1	0.5	0	0.0
Positive control EMS																			
500 μg/ml	A	100	9.2	11	22.0	11	22.0	8	16.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	9.7	12	24.0	12	24.0	10	20.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	200	9.5	23	23.0**	23	23.0**	18	18.0**	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

From Schulz & Landsiedel (2009g)

DMSO, dimethyl sulfoxide; EMS, ethylmethanesulfonate; ** $P \leq 0.01$ (Fisher's exact test, one-sided, with Bonferoni-Holm corrections)

Table 159. Chromosomal aberration test with M700F048 with metabolic activation (4-hour treatment, harvest after 28 hours): Experiment 2

	Culture	No. of metaphases	Mitotic index	Aberrant cells including gaps		Aberrant cells excluding gaps		Exchange figures		Multiple aberrations		Chromosome disintegrations		Aneuploidy		Polyploidy		Endopolyploidy	
		n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Vehicle DMSO	A	100	13.2	7	7.0	5	5.0	3	3.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	8.6	6	6.0	3	3.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	200	10.9	13	6.5	8	4.0	4	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
M700F048																			
500 µg/ml	A	100	15.4	5	5.0	4	4.0	3	3.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	10.9	3	3.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	200	13.2	8	4.0	4	2.0	3	1.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
750 µg/ml	A	100	17.9	2	2.0	2	2.0	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	11.3	3	3.0	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	200	14.6	5	2.5	4	2.0	2	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
1000 µg/ml	A	50	6.4	13	26.0	12	24.0	1	2.0	0	0.0	0	0.0	0	0.0	1	2.0	0	0.0
	B	50	11.5	10	20.0	10	20.0	4	8.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	100	9.0	23	23.0**	22	22.0**	5	5.0	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0
Positive control CP																			
0.5 µg/ml	A	50	19.2	9	18.0	9	18.0	5	10.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	50	21.3	10	20.0	10	20.0	6	12.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	100	20.3	19	19.0**	19	19.0**	11	11.0**	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

From Schulz & Landsiedel (2009g)

CP, cyclophosphamide; DMSO, dimethyl sulfoxide; ** $P \leq 0.01$ (Fisher's exact test, one-sided, with Bonferoni-Holm corrections)

Table 160. Chromosomal aberration test with M700F048 with metabolic activation (4-hour treatment, harvest after 28 hours): Experiment 3

	Culture	No. of metaphases	Mitotic index	Aberrant cells including gaps		Aberrant cells excluding gaps		Exchange figures		Multiple aberrations		Chromosome disintegrations		Aneuploidy		Polyploidy		Endopolyploidy	
		n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Vehicle DMSO	A	100	18.6	8	8.0	4	4.0	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	12.1	8	8.0	3	3.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	200	15.4	16	8.0	7	3.5	3	1.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
M700F048																			
800 µg/ml	A	100	9.1	4	4.0	3	3.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	12.0	7	7.0	4	4.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	200	10.6	11	5.5	7	3.5	1	0.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
1000 µg/ml	A	100	5.7	10	10.0	10	10.0	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	12.1	13	13.0	9	9.0	4	4.0	0	0.0	0	0.0	0	0.0	0	0.0	2	2.0
	A + B	200	8.9	23	11.5	19	9.5*	6	3.0	0	0.0	0	0.0	0	0.0	0	0.0	2	1.0
1200 µg/ml	A	50	8.2	9	18.0	8	16.0	5	10.0	0	0.0	0	0.0	0	0.0	0	0.0	2	3.8
	B	50	14.0	10	20.0	8	16.0	2	4.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	100	11.1	19	19.0*	16	16.0*	7	7.0	0	0.0	0	0.0	0	0.0	0	0.0	2	2.0
Positive control CP																			
0.5 µg/ml	A	50	12.6	9	18.0	9	18.0	5	10.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	50	15.7	17	34.0	16	32.0	9	18.0	1	2.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	100	14.2	26	26.0**	25	25.0**	14	14.0**	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0

From Schulz & Landsiedel (2009g)

CP, cyclophosphamide; DMSO, dimethyl sulfoxide; * $P \leq 0.05$; ** $P \leq 0.01$ (Fisher's exact test, one-sided, with Bonferoni-Holm corrections)

In an in vivo bone marrow micronucleus assay, Reg. No. 5570265 (purity 96.1%) prepared in suspension in DMSO/corn oil (ratio 2:3) was administered via gavage to NMRI mice (five males per dose) once at a dose level of 0 (vehicle), 500, 1000 or 2000 mg/kg bw per day. The vehicle served as negative control and cyclophosphamide and vincristine sulfate as positive controls. The animals were sacrificed 24 or 48 (additional high-dose and vehicle groups) hours after the administration, and the bone marrow of the two femora was prepared. After staining of the preparations, 2000 polychromatic erythrocytes were evaluated per animal and investigated for micronuclei. The normocytes with and without micronuclei occurring per 2000 polychromatic erythrocytes were also recorded.

There were no deaths, no treatment-related clinical signs and no apparent suppression of body weight gain at any dose level. The bioavailability of the test substance after an oral application was confirmed in a separate study (Fabian & Landsiedel, 2009c). The group mean frequencies of micronucleated polychromatic erythrocytes in the treated groups were similar to the vehicle control frequency, and none was statistically significantly different from the control frequency. There was no significant difference in the proportion of polychromatic erythrocytes relative to total erythrocytes between any of the Reg. No. 5435595–treated groups and the negative control group. The frequencies of micronucleated polychromatic erythrocytes and the proportion of polychromatic erythrocytes in both the vehicle control and positive control groups were within the range of historical control data for the performing laboratory. Both positive control chemicals (i.e. cyclophosphamide for clastogenic effects and vincristine sulfate for induction of spindle poison effects) led to the expected increase in the rate of polychromatic erythrocytes containing small (cyclophosphamide) or small and large (vincristine sulfate) micronuclei, thus demonstrating the sensitivity of the test system.

It was concluded that Reg. No. 5570265 did not induce the formation of micronucleated polychromatic erythrocytes in male mouse bone marrow cells under the conditions employed for this study at the maximum dose levels specified in the guidelines (Schulz & Landsiedel, 2009i).

In an in vivo unscheduled DNA synthesis assay, Reg. No. 5570265 (purity 96.1%) prepared in suspension in DMSO/corn oil (ratio 2:3) was administered via gavage to Wistar rats (three males per dose) once at a dose level of 0 (vehicle), 1000 or 2000 mg/kg bw. As positive control, 2-acetylaminofluorene was administered at a dose of 50 mg/kg bw. Hepatocytes were harvested 3 and 14 hours after administration. No increases in the number of net nuclear grain counts or in the percentage of cells in repair were noted at any dose in the hepatocytes harvested 3 or 14 hours after a single oral administration of M700F048. In contrast, 2-acetylaminofluorene treatment led to a marked increase in the number of net nuclear grains as well as the percentage of cells in repair, thus demonstrating the sensitivity of the test system.

Thus, under the experimental conditions of this assay, Reg. No. 5570265 is considered to be negative in the in vivo unscheduled DNA synthesis assay using rat hepatocytes (Schulz & Landsiedel, 2009e).

In a developmental toxicity study in rabbits, Reg. No. 5570265 (purity 96.1%) was administered to mated female New Zealand White rabbits (31 or 32 per dose) via gavage, from day 6 to day 28 of gestation, at a dose level of 0, 10, 30 or 100 mg/kg bw per day at 10 ml/kg bw in aqueous carboxymethylcellulose (1% w/v). The animals were killed on day 29 after mating for reproductive assessment and fetal examination. Clinical signs, body weight and feed consumption were recorded. Adult females were examined macroscopically at necropsy on day 29 after mating, and all fetuses were examined macroscopically at maternal necropsy and subsequently by detailed internal visceral and skeletal examination.

The high dose of 100 mg/kg bw per day elicited signs of maternal toxicity, as indicated by clinical signs (no defecation), five cases of abortion (Tables 161 and 162) and decreased feed consumption (up to −46% on individual days, −15% during the treatment period), corroborated by statistically significant decreases in body weight gain during gestation days 11–14, 14–16 and 16–19 as well as a decrease in cumulative body weight gain by about 35% during the treatment period

(gestation days 6–28) (Tables 163 and 164) and stomach erosions in two dams (Table 162). No treatment-related effects on caesarean section parameters were observed at any dose (Table 165).

Table 161. Clinical observations in rabbits administered M700F048 during days 6–28 of gestation

Dose (mg/kg bw per day)	Animal no.	Gestation day(s)	Observation
0 (control)	17	29	Abortion—sacrificed
	20	24–29	No defecation
	28	22	Found dead
10	46	9–21 21	No defecation Abortion—sacrificed
	54	28	Abortion—sacrificed
	60	20	Abortion—sacrificed
30	84	21	Abortion—sacrificed
100	103	19–23	No defecation
	107	19–23 23	No defecation Abortion—sacrificed
	109	24–26 26	No defecation Abortion—sacrificed
	111	19–21	No defecation
	114	28	Abortion—sacrificed
	117	22–29 29	No defecation Abortion—sacrificed
	121	21–28 28	No defecation Abortion—sacrificed

From Schneider (2010)

Table 162. Gross necropsy findings in rabbits administered M700F048 during days 6–28 of gestation

Dose (mg/kg bw per day)	Animal no.	Observation
0 (control)	12	Blind ending uterine horn (unilateral)
	26, DGE	Thoracic cavity filled with blood
10	41, DGE	Thoracic cavity filled with test substance
	45	Blind ending uterine horn (unilateral)
	46, AS	Stomach filled with very dry, hard feed; watery faeces
	48	Absent uterine horn (unilateral)
	54, AS	Single stomach erosions; watery faeces
30	84, AS	No faeces in rectum
100	107, AS	Watery faeces
	109, AS	Watery faeces
	114, AS	Single stomach erosions; watery faeces
	119, NP	Distended bladder, massively dilated and filled with urine and white concrement
	121, AS	Single stomach erosions; watery faeces

From Schneider (2010)
AS, aborted, sacrificed; DGE, died after gavage error; NP, not pregnant

Table 163. Feed consumption and body weight development in rabbits administered M700F048 during days 6–28 of gestation

	Mean value ± SD (*n*)			
	0 mg/kg bw per day	10 mg/kg bw per day	30 mg/kg bw per day	100 mg/kg bw per day
Feed consumption (g/animal per day)[a]				
Days 1–6	147.3 ± 36.74 (5)	142.3 ± 43.82 (5)	143.7 ± 54.28 (5)	144.7 ± 47.26 (5)
% change[b]	—	*−3.4*	*−2.4*	*−1.8*
Days 6–28	142.9 ± 33.78 (22)	144.4 ± 24.60 (22)	144.4 ± 29.82 (22)	120.9 ± 35.82 (22)
% change[b]	—	*+1.0*	*+1.0*	*−15.4*
Days 1–29	141.7 ± 34.65 (28)	142.3 ± 28.86 (28)	142.3 ± 35.07 (28)	124.1 ± 38.18 (28)
% change[b]	—	*+0.4*	*+0.4*	*−12.4*
Body weight gain (g)[c]				
Days 0–6	108.0 (28)	105.7 (27)	144.3 (30)	138.7 (27)
% change[b]	—	*−2.1*	*+33.6*	*+28.4*
Days 6–28	352.2 (27)	408.3 (24)	342.9 (29)	227.5* (23)
% change[b]	—	*+15.9*	*−2.6*	*−35.4*
Days 0–29	492.8 (26)	542.3 (24)	524.2 (29)	417.9 (22)
% change[b]	—	*+10.0*	*+6.4*	*−15.2*

From Schneider (2010)
SD, standard deviation; * $P \leq 0.05$ (Dunnett's test, two-sided)
[a] Mean of means.
[b] Compared with controls.
[c] Standard deviations not provided in study report.

Table 164. Mean gravid uterus weights and net body weight change of pregnant rabbits administered M700F002 during days 6–28 of gestation

	Mean value ± SD			
	0 mg/kg bw per day	10 mg/kg bw per day	30 mg/kg bw per day	100 mg/kg bw per day
Gravid uterus weight (g)	491.9 ± 82.83	500.0 ± 103.34	525.9 ± 118.17	473.4 ± 88.88
Carcass weight (g)	3648.8 ± 327.37	3733.5 ± 281.98	3643.2 ± 325.02	3496.6 ± 351.48
Net weight change from day 6 (g)	−101.1 ± 199.47	−67.6 ± 203.64	−145.5 ± 156.02	−198.0 ± 186.39

From Schneider (2010)
SD, standard deviation

Table 165. Pregnancy status and caesarean section data for does administered M700F048 during days 6–28 of gestation

	0 mg/kg bw per day	10 mg/kg bw per day	30 mg/kg bw per day	100 mg/kg bw per day
Pregnancy status				
Mated (*n*)	32	31	31	31
Pregnant (*n*)	29	28	30	28
Conception rate (%)	91	90	97	90

Table 165 (continued)

	0 mg/kg bw per day	10 mg/kg bw per day	30 mg/kg bw per day	100 mg/kg bw per day
Aborted (*n*)	1	3	1	5
Premature birth (*n*)	0	0	0	0
Dams with viable fetuses (*n*)	26	24	29	22
Dams with all resorptions (*n*)	0	0	0	0
Mortality (*n*)	3	4	1	6
Pregnant at terminal sacrifice (*n*)	26	24	29	22
Caesarean section data[a]				
Corpora lutea (*n*)	10.8 ± 1.63	11.0 ± 2.32	11.8 ± 2.44	11.4 ± 1.76
- total number (*n*)	281	264	342	251
Implantation sites (*n*)	9.5 ± 1.86	9.5 ± 2.69	10.8 ± 2.68	10.2 ± 1.82
- total number (*n*)	248	227	312	224
Preimplantation loss (%)	11.2 ± 13.68	13.5 ± 18.20	9.0 ± 11.21	10.3 ± 11.30
Post-implantation loss (%)	6.4 ± 7.35	6.5 ± 8.16	11.0 ± 12.61	10.7 ± 10.25
Resorptions (*n*)	0.6 ± 0.75	0.7 ± 0.87	1.0 ± 1.05	1.2 ± 1.26
- total number (*n*)	16	16	30	26
Early resorptions (%)	4.0 ± 5.89	3.5 ± 6.00	7.1 ± 9.62	3.5 ± 6.79
- number (*n*)	0.4 ± 0.57	0.4 ± 0.65	0.6 ± 0.78	0.4 ± 0.73
- total number (*n*)	10	9	18	8
Late resorptions (%)	2.4 ± 4.50	3.0 ± 6.50	3.8 ± 7.99	7.2 ± 9.51
- number (*n*)	0.2 ± 0.43	0.3 ± 0.62	0.4 ± 0.78	0.8* ± 1.22
- total number (*n*)	6	7	12	18
Dead fetuses (*n*)	0	0	0	0
Dams with viable fetuses (*n*)	26	24	29	22
Live fetuses	8.9 ± 1.87	8.8 ± 2.52	9.7 ± 3.02	9.0 ± 1.41
- total number (*n*)	232	211	282	198
Total live female fetuses (*n*)	4.3 ± 1.65	4.8 ± 1.95	4.8 ± 2.13	4.3 ± 1.86
- total number (*n*)	113	116	138	95
- mean (%)	45.8 ± 15.55	52.6 ± 18.77	44.8 ± 18.24	42.5 ± 16.39
Total live male fetuses (*n*)	4.6 ± 1.84	4.0 ± 1.63	5.0 ± 2.72	4.7 ± 1.81
- total number (*n*)	119	95	144	103
- mean (%)	47.8 ± 15.29	40.8 ± 14.98	44.2 ± 19.52	46.8 ± 17.65
% live females	48.7	55.0	48.9	48.0
% live males	51.3	45.0	51.1	52.0
Placental weights (g)	5.0 ± 0.61	5.3 ± 0.95	5.1 ± 0.77	4.6 ± 0.53
- male fetuses (g)	5.2 ± 0.63	5.3 ± 0.92	5.1 ± 0.79	4.7 ± 0.67
- female fetuses (g)	4.8 ± 0.77	5.2 ± 0.96	5.1 ± 0.83	4.5 ± 0.75
Mean fetal weight (g)	37.0 ± 4.02	38.5 ± 4.30	37.2 ± 4.52	34.9 ± 4.35
- males (g)	37.4 ± 4.00	38.3 ± 4.69	37.4 ± 5.08	35.6 ± 4.46
- females (g)	36.3 ± 4.71	37.9 ± 4.52	37.2 ± 4.42	34.3 ± 5.93

From Schneider (2010)

* $P \leq 0.05$; ** $P \leq 0.01$ (Dunnett's test, two-sided)

[a] Mean ± standard deviation on a litter basis.

Fetal examination did not reveal any treatment-related external, visceral or skeletal malformations, variations or unclassified observations.

The maternal NOAEL was 30 mg/bw kg per day, based on increased mortality, stomach erosions, abortions and late resorptions as well as decreased body weight gain at 100 mg/kg bw per day. The developmental NOAEL was 30 mg/bw kg per day, based on increased abortions and late resorptions at 100 mg/kg bw day (Schneider, 2010).

3. Observations in humans

Medical data on fluxapyroxad are limited, but no reports of adverse effects were identified during routine monitoring of production plant workers or among personnel involved in the experimental biological testing or field trials. There is no evidence or data available to support any findings in relation to poisoning with fluxapyroxad.

Comments

Biochemical aspects

In rats, ^{14}C-labelled fluxapyroxad was rapidly and moderately well absorbed from the gastrointestinal tract following oral dosing. The extent of absorption was approximately 65–80% of the administered dose, independent of dose and sex. Maximum concentrations of radioactivity in plasma were observed within 1 hour of dosing for the low-dose group (5 mg/kg bw), 8 hours for the mid-dose group (50 mg/kg bw) and 24 hours for the high-dose group (500 mg/kg bw). In another study in rats, maximum tissue concentrations occurred within 1 hour post-dosing at the low dose (7.5 mg/kg bw) and 16 hours post-dosing at the high dose (150 mg/kg bw), with higher concentrations of radioactivity found in liver, thyroid and adrenals. Very little fluxapyroxad was present in other tissues at the end of the study (7 days). There were no major sex-related differences in the pattern of excretion. Faecal excretion was the primary route of elimination, and excretion was rapid, with the majority of the administered dose (61–83%) excreted by all routes within 48 hours after dosing.

The main biotransformation mechanisms of fluxapyroxad in rats are hydroxylation at the biphenyl ring, *N*-demethylation at the pyrazole ring, loss of a fluorine atom at the biphenyl ring and conjugation with glucuronic acid or glutathione, yielding about 50 metabolites.

Toxicological data

The LD_{50} in rats treated orally or dermally with fluxapyroxad was greater than 2000 mg/kg bw. The LC_{50} in rats treated by inhalation was greater than 5.1 mg/l of air. Fluxapyroxad was minimally irritating to the skin of rabbits, not irritating to the eyes of rabbits and not sensitizing in guinea-pigs under the conditions of the maximization test.

Following repeated gavage or dietary dosing, the liver was the main target organ in mice, rats and dogs. In general, the main effects in mice and rats were increased liver weight, liver enlargement and centrilobular hepatocellular hypertrophy, as well as alterations in clinical chemistry. In the dog, increased liver weights and alterations in clinical chemistry were accompanied by fibrosis. The thyroid was also a target in mice and rats, with effects including increased thyroid weight, changes in hormone levels (T_4 and TSH) and thyroid follicular cell hypertrophy and hyperplasia. Other treatment-related effects at higher doses consisted of siderosis and impaired iron storage in rats and dogs, as well as teeth whitening and shortened prothrombin time in rats only.

The NOAEL in a 90-day mouse study was 2000 ppm (equal to 390 mg/kg bw per day), based on decreased body weight and multifocal necrosis in the liver in males at 6000 ppm (equal to 1136 mg/kg bw per day). The NOAEL in a 90-day rat study was 100 ppm (equal to 7.3 mg/kg bw per day), based on liver and thyroid effects in females (increased absolute and relative liver weights, increased incidences of centrilobular hepatocellular hypertrophy and hypertrophy/hyperplasia of thyroid follicular cells) at 500 ppm (equal to 35.1 mg/kg bw per day). The NOAEL in a 1-year dog study was

300 ppm (equal to 9 mg/kg bw per day), based on clinical chemistry alterations and fibrosis in the liver in females at 1500 ppm (equal to 43 mg/kg bw per day).

In an 18-month carcinogenicity study in mice, the NOAEL was 750 ppm (equal to 107 mg/kg bw per day), based on decreased body weight gain at 3000 ppm (equal to 468 mg/kg bw per day). There was no evidence of carcinogenicity in mice.

In a 2-year rat study, the NOAEL was 50 ppm (equal to 2.1 mg/kg bw per day), based on reduced body weight gain in the absence of an effect on feed consumption at 250 ppm (equal to 11 mg/kg bw per day). The incidence of combined hepatocellular adenomas and carcinomas in males was increased at the top two doses of 1500 ppm (equal to 68 mg/kg bw per day) and 3000 ppm (equal to 145 mg/kg bw per day), and there was an increased incidence of hepatocellular adenomas in females at the highest dose (3000 ppm, equal to 145 mg/kg bw per day); this incidence also slightly exceeded the historical control range. There was a small increase in the incidence of thyroid follicular cell adenomas and carcinomas in males at the highest dose tested; this incidence was within the historical control range (4–30%), but above the historical control mean (15%). The incidence and severity of follicular cell hyperplasia were increased starting at 1500 ppm (equal to 68 mg/kg bw per day). The Meeting concluded that high doses of fluxapyroxad caused an increased incidence of hepatocellular adenomas and carcinomas in males, hepatocellular adenomas in females and follicular cell adenomas and carcinomas combined in the thyroid in males.

Special studies were conducted to examine liver effects in the rat. These studies showed that fluxapyroxad increased microsomal protein levels and cytochrome P450 activity, specifically BROD and PROD, and cell proliferation in the liver of rats. The Meeting concluded that for the liver tumours in rats, there was sufficient evidence to support the proposed mitogenic mode of action associated with induction of CYP2B-type cytochrome P450. Special studies on the mode of action in the thyroid produced equivocal results.

Fluxapyroxad was adequately tested for genotoxicity in vitro and in vivo in a range of assays and was not found to be genotoxic.

The Meeting concluded that fluxapyroxad was unlikely to be genotoxic.

On the basis of the lack of genotoxicity, the absence of carcinogenicity in mice and the presence of liver and thyroid follicular cell tumours in rats only at high doses, the Meeting concluded that fluxapyroxad is unlikely to pose a carcinogenic risk to humans at anticipated dietary residue levels.

No effects on reproduction were noted in a multigeneration reproductive toxicity study in the rat. However, there was a decrease in body weight and increased incidences of hepatocellular hypertrophy and thyroid follicular cell hypertrophy/hyperplasia in the offspring in both generations at 50 mg/kg bw per day and above. The marginal increase in the incidence of very slight hepatocellular hypertrophy at 10 mg/kg bw per day in F_1 offspring was not considered to be toxicologically relevant. At the high dose only, whitening of the incisors due to a decrease in iron-containing pigment in ameloblasts was observed. The NOAEL for parental toxicity was 10 mg/kg bw per day, based on decreased body weight gain and effects on the liver and thyroid at 50 mg/kg bw per day. The NOAEL for offspring toxicity was 10 mg/kg bw per day, based on reduced body weight and body weight gain and liver effects at 50 mg/kg bw per day. The NOAEL for reproductive toxicity was 300 mg/kg bw per day, the highest dose tested.

In a developmental toxicity study in rats, there were no effects on development observed when pregnant rats were administered doses up to 1000 mg/kg bw per day. There was a transient decrease in body weight gain from gestation day 6 to gestation day 8 in dams at and above 200 mg/kg bw per day. Increased liver and thyroid weights and increased incidences of thyroid follicular cell hypertrophy/hyperplasia were observed in maternal animals at 1000 mg/kg bw per day. The NOAEL for maternal toxicity in rats was 25 mg/kg bw per day, and the NOAEL for developmental toxicity was 1000 mg/kg bw per day, the highest dose tested. In rabbits, there was an increase in early resorptions, as well as one abortion, and a decrease in fetal weight at the high dose (60 mg/kg bw per day), which occurred in the presence of a marked reduction in feed consumption and body weight.

Fetal weights were also reduced, and there was an increased incidence of paw hyperflexion at the high dose. The NOAEL for maternal and developmental toxicity in rabbits was 25 mg/kg bw per day.

The Meeting concluded that fluxapyroxad was not teratogenic in rats or rabbits.

In an acute neurotoxicity study in rats, the NOAEL was 125 mg/kg bw, based on decreased motor activity and rearing at a dose of 500 mg/kg bw. There was no histological evidence of damage to the central or peripheral nervous system. There was no evidence of neurotoxicity in a 90-day neurotoxicity study in rats.

In a 4-week immunotoxicity study in mice, no adverse effects were observed at any dose up to 6000 ppm (equal to 1323 mg/kg bw per day), the highest dose tested.

Three minor metabolites in rats that are also found in plants and soil were assessed for toxicity. The oral LD_{50} in rats for the metabolite M700F001 was greater than 2000 mg/kg bw. In a 90-day feeding study in rats, the NOAEL for M700F001 was 954 mg/kg bw per day, the highest dose tested. The metabolite was not genotoxic in any of an adequate range of in vitro and in vivo genotoxicity assays. In a developmental toxicity study in rabbits, the maternal and developmental NOAEL was 250 mg/kg bw per day, the highest dose tested.

The oral LD_{50} in rats for the metabolite M700F002 was greater than 2000 mg/kg bw. In a 28-day dietary study of M700F002 in rats, the NOAEL was 1165 mg/kg bw per day, the highest dose tested. In a 90-day feeding study in rats, the NOAEL for M700F002 was 929 mg/kg bw per day, the highest dose tested. This metabolite was not genotoxic in any of an adequate range of in vitro and in vivo genotoxicity assays. In a developmental toxicity study in rabbits, the maternal and developmental NOAELs were 300 mg/kg bw per day, based on increased maternal mortality, abortions and stomach erosions as well as decreased body weight gain at 1000 mg/kg bw per day.

The oral LD_{50} in rats for the metabolite M700F048 was greater than 2000 mg/kg bw. In a 28-day dietary study of M700F048 in rats, the NOAEL was 189 mg/kg bw per day, based on decreased body weight gain at 1478 mg/kg bw per day. This metabolite was not genotoxic in any of an adequate range of in vitro and in vivo genotoxicity assays. In a developmental toxicity study in rabbits, the maternal and developmental NOAEL was 30 mg/kg bw per day, based on increased maternal mortality, abortions, late resorptions and stomach erosions as well as decreased body weight gain at 100 mg/kg bw per day.

The metabolites were not considered to be more toxic than fluxapyroxad.

There was no information available on adverse health effects in manufacturing plant personnel or in operators and workers exposed to fluxapyroxad formulations during their use. There are no reports of poisoning with fluxapyroxad.

The Meeting concluded that the existing database on fluxapyroxad was adequate to characterize the potential hazards to fetuses, infants and children.

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) of 0–0.02 mg/kg bw on the basis of the NOAEL of 2.1 mg/kg bw per day in the 2-year rat combined chronic toxicity/carcinogenicity study for body weight effects in both sexes in the absence of effects on feed consumption. A safety factor of 100 was applied.

The Meeting established an acute reference dose (ARfD) of 0.3 mg/kg bw on the basis of the NOAEL of 25 mg/kg bw per day in the developmental toxicity study in rabbits for early resorptions and the rat developmental toxicity study based on a transient decrease in body weight gain from gestation day 6 to gestation day 8. A safety factor of 100 was applied.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen-month study of toxicity and carcinogenicity[a]	Toxicity	750 ppm, equal to 107 mg/kg bw per day	3000 ppm, equal to 468 mg/kg bw per day
		Carcinogenicity	6000 ppm, equal to 996 mg/kg bw per day[b]	—
Rat	Two-year study of toxicity and carcinogenicity[a]	Toxicity	50 ppm, equal to 2.1 mg/kg bw per day	250 ppm, equal to 11 mg/kg bw per day
		Carcinogenicity	250 ppm, equal to 11 mg/kg bw per day	1500 ppm, equal to 68 mg/kg bw per day
	Two-generation study of reproductive toxicity[a]	Reproductive toxicity	300 mg/kg bw per day[b]	—
		Parental toxicity	10 mg/kg bw per day	50 mg/kg bw per day
		Offspring toxicity	10 mg/kg bw per day	50 mg/kg bw per day
	Developmental toxicity study[c]	Maternal toxicity	25 mg/kg bw per day	200 mg/kg bw per day
		Embryo and fetal toxicity	1000 mg/kg bw per day[b]	—
Rabbit	Developmental toxicity study[c]	Maternal toxicity	25 mg/kg bw per day	60 mg/kg bw per day
		Embryo and fetal toxicity	25 mg/kg bw per day	60 mg/kg bw per day
Dog	One-year study of toxicity[a]	Toxicity	300 ppm, equal to 9 mg/kg bw per day	1500 ppm, equal to 43 mg/kg bw per day

[a] Dietary administration.
[b] Highest dose tested.
[c] Gavage administration.

Estimate of acceptable daily intake for humans

0–0.02 mg/kg bw

Estimate of acute reference dose

0.3 mg/kg bw

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposure

Critical end-points for setting guidance values for exposure to fluxapyroxad

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid; to a moderate extent
Dermal absorption	Not available
Distribution	Widely distributed; highest concentrations in liver, thyroid and adrenals
Potential for accumulation	No evidence of accumulation
Rate and extent of excretion	Largely complete within 24 h; primarily via faeces (70–85%, bile 30–54%) and to a lesser extent urine (8–17%)
Metabolism in animals	Extensive
Toxicologically significant compounds in animals, plants and the environment	Parent compound, M007F048
Acute toxicity	
Rat, LD_{50}, oral	> 2000 mg/kg bw
Rat, LD_{50}, dermal	> 2000 mg/kg bw
Rat, LC_{50}, inhalation	> 5.1 mg/l
Rabbit, dermal irritation	Minimally irritating
Rabbit, ocular irritation	Not irritating
Dermal sensitization	Not sensitizing (Magnusson & Kligman)
Short-term studies of toxicity	
Target/critical effect	Liver (clinical chemistry changes), thyroid (hypertrophy/hyperplasia)
Lowest relevant oral NOAEL	7.3 mg/kg bw per day (rats)
Lowest relevant dermal NOAEL	1000 mg/kg bw per day (rats)
Lowest relevant inhalation NOAEC	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Body weight
Lowest relevant NOAEL	2.1 mg/kg bw per day (rats)
Carcinogenicity	Liver and thyroid tumours observed in rats at high doses; unlikely to pose a carcinogenic risk to humans at anticipated dietary intake levels
Genotoxicity	
	Not genotoxic
Reproductive toxicity	
Reproduction target/critical effect	No effect on fertility at highest dose tested; decrease in body weight, liver and thyroid effects in pups at parentally toxic dose
Lowest relevant parental NOAEL	10 mg/kg bw per day
Lowest relevant reproductive NOAEL	300 mg/kg bw per day (highest dose tested)
Lowest relevant offspring NOAEL	10 mg/kg bw per day
Developmental toxicity	
Developmental target/critical effect	Decreased fetal weight and paw hyperflexion at maternally toxic dose (rabbits)
Lowest relevant maternal NOAEL	25 mg/kg bw per day (rats and rabbits)
Lowest relevant developmental NOAEL	25 mg/kg bw per day (rabbits)

Neurotoxicity	
Target/critical effect	Decreased motor activity and rearing
Acute neurotoxicity NOAEL	125 mg/kg bw (rats)
Immunotoxicity	
	1323 mg/kg bw per day (highest dose tested; mice)
Other toxicological studies	
	Toxicity studies on metabolites Special studies on liver and thyroid tumour modes of action
Medical data	
	No reports received

Summary

	Value	Study	Safety factor
ADI	0–0.02 mg/kg bw	Two-year toxicity/carcinogenicity study (rat)	100
ARfD	0.3 mg/kg bw	Developmental toxicity studies (rat and rabbit)	100

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Appendix 1: Summary of identified metabolites in urine, faeces, bile, liver, kidney and plasma

Metabolite designation (code)	Relative molecular mass	Structure	Urine	Faeces	Bile	Liver	Kidney	Fat	Plasma
Fluxapyroxad = M700F000	381		−	+	−	+	+	+	+
M700F001	176		+	−	−	−	−	−	−
M700F002	162		+	−	−	−	−	−	−
M700F004	573		+	−	+	−	−	−	+
M700F005	397		+	+	+	+	+	−	+
M700F006	397		−	+	+	+	+	−	+
M700F008	367		−	+	−	+	+	+	+
M700F009	383		+	+	+	+	+	−	+

Metabolite designation (code)	Relative molecular mass	Structure	Urine	Faeces	Bile	Liver	Kidney	Fat	Plasma
M700F010	383	F, F, O, N, N, H, N, H, OH, F, F, F	−	+	−	+	+	−	+
M700F011	571	F, F, O, N, N, N, H, O-GlcA, OH, F, F	+	−	−	−	−	−	+
M700F012	559	F, F, O, N, N, H, N, H, O-GlcA, F, F, F	+	−	−	−	−	−	+
M700F013	573	F, F, O, N, N, N, H, O-GlcA, F, F, F	+	−	−	−	−	−	+
M700F014	559	F, F, O, N, N, H, N, H, O-GlcA, F, F, F	+	−	+	−	−	−	+
M700F015	555	F, F, O, N, N, N, H, O-GlcA, F, F	+	−	+	−	−	−	+
M700F016	443	F, F, O, N, N, N, H, SCH_3, OH, F, F, F	+	+	−	+	−	−	−
M700F019	573	F, F, O, N, N, N, H, O-GlcA, F, F, F	+	−	−	−	−	−	−

Metabolite designation (code)	Relative molecular mass	Structure	Urine	Faeces	Bile	Liver	Kidney	Fat	Plasma
M700F020	589		+	–	–	–	–	–	–
M700F021	541		+	–	–	–	–	–	–
M700F022	559		+	–	–	–	–	–	–
M700F023	559		+	–	–	–	–	–	–
M700F024	379		+	+	+	+	–	–	–
M700F025	463		+	–	–	+	–	–	–
M700F026	477		+	–	–	+	–	–	–
M700F027	459		+	–	–	–	–	–	–

Metabolite designation (code)	Relative molecular mass	Structure	Urine	Faeces	Bile	Liver	Kidney	Fat	Plasma
M700F028	619	F, F, O, N, N, N, H, O-GlcA, SCH_3, F, F, F	+	–	–	–	–	–	–
M700F029	605	F, F, O, N, N, N, H, O-GlcA, SCH_3, F, F, F	+	–	–	–	–	–	–
M700F032	361	HOOC, O, N, N, N, H, F, F, F	–	–	+	–	–	–	–
M700F042	397	F, F, O, N, N, N, H, OH, F, F, F	–	–	+	–	–	–	–
M700F050	559	F, F, O, N, N, N, H, H, O-GlcA, F, F, F	–	–	–	–	+	–	–
M700F061	559	F, F, O, N, N, N, H, H, O-GlcA, F, F, F	+	–	–	–	–	–	–
M700F063	498	F, F, O, N, N, N, H, S, COOH, OH, NH_2, F, F	–	–	–	–	+	–	–
M700F102	445	F, F, O, N, N, N, H, H, SCH_3, OH, OH, F, F, F	–	–	–	+	–	–	–

Metabolite designation (code)	Relative molecular mass	Structure	Urine	Faeces	Bile	Liver	Kidney	Fat	Plasma
M700F103	429		–	–	–	+	–	–	–
M700F104	445		–	–	–	+	–	–	–
M700F105	427		–	–	–	+	–	–	–
M700F106	425		–	–	–	+	–	–	–
M700F107	443		–	–	–	–	–	–	+
M700F108	425		–	–	–	+	–	–	–
M700F113	555		–	–	+	–	–	–	–

Metabolite designation (code)	Relative molecular mass	Structure	Urine	Faeces	Bile	Liver	Kidney	Fat	Plasma
M700F114	674		−	−	+	−	−	−	−
M700F115	555		−	−	+	−	−	−	−
M700F116	502		−	−	+	−	−	−	−
M700F117	498		−	−	+	−	−	−	−
M700F118	516		−	−	+	−	−	−	−
M700F119	498		−	−	+	−	−	−	−
M700F120	500		−	−	+	−	−	−	−

Metabolite designation (code)	Relative molecular mass	Structure	Urine	Faeces	Bile	Liver	Kidney	Fat	Plasma
M700F121	498	F, F, O, N, N, N, H, S, COOH, OH, NH2, F, F	–	–	+	–	–	–	–
M700F122	486	F, F, O, N, N, H, N, H, S, COOH, NH2, F, F, F	–	–	+	–	–	–	–
M700F123	559	F, F, O, N, N, H, N, H, O-GlcA, F, F, F	–	–	+	–	–	–	–
M700F124	559	F, F, O, N, N, N, H, OGlcA, F, F, F	–	–	+	–	–	–	–
M700F125	573	F, F, O, N, N, N, H, OGlcA, F, F, F	–	–	+	–	–	–	–

Appendix 2: Application of the IPCS conceptual framework for cancer risk assessment

A1. Introduction

Administration of fluxapyroxad was associated with statistically significant increases in follicular cell adenomas and combined adenomas and carcinomas in the livers of male rats at doses of 1500 ppm and above. In female rats at the high dose, the incidence of adenomas was higher than in the controls (see Table 68 in section 2.3). There was also a statistically significant increase in combined follicular cell adenomas and carcinomas in the thyroid in male rats at the highest dose tested, with a non-statistically significant increase at 1500 ppm. In mice, no treatment-related thyroid tumours were observed in either sex at any dose. The data relating to the postulated mode of action (MOA) by which fluxapyroxad induces liver and thyroid tumours in rats are summarized in this appendix. The analytical approach applied to the postulated MOA was based on the International Programme on Chemical Safety (IPCS) conceptual framework for evaluating an MOA for chemical carcinogens (Sonich-Mullin et al., 2001; Boobis et al., 2006).

A2. Postulated mode of action (liver)

The MOA for liver tumours induced by fluxapyroxad was mitogenesis in the context of key events that include enzyme induction, hepatocellular hypertrophy and non-neoplastic alterations in the liver at the gross and microscopic levels, ultimately resulting in the emergence of adenomas and carcinomas.

A2.1 Key events in the mode of action (liver)

A2.1.1 Liver enzyme induction

Fluxapyroxad administration for 14 days caused increases in total liver CYP and induction of PROD/BROD, which are associated with CYP2B in particular. This occurs at doses of 250 ppm and above. Total liver CYP and PROD/BROD levels return to levels equal to those of controls (or slightly higher) in a 4-week recovery group, indicating near to complete reversibility of this effect (Buesen et al., 2009e).

A2.1.2 Cell proliferation

Cell proliferation in the liver was measured by labelling with 5-bromo-2′-deoxyuridine (BrdU) (Buesen et al., 2010b,c,d). A dose- and time-related increase occurs in all liver zones (1, 2 and 3), but was highest in zone 3, the centrilobular region. At doses of 1500 ppm and above, increased cell proliferation was maximal in males at days 3–7 and negligible by day 28. In females, increased cell proliferation was observed at doses of 250 ppm and higher from day 3 to day 91, was maximal on day 7 and declined by days 28 and 91, but remained significant during this period. Treatment for 28 days with fluxapyroxad followed by a 28-day recovery period did not result in significant cell proliferation at any dose level (Buesen et al., 2010b,c,d). As a result, reversibility of cell proliferation following cessation of treatment was not demonstrated. However, maximum cell proliferation occurred at day 7 in both males and females. By day 28, cell proliferation was essentially zero in males and about 30% of the maximum in females.

A2.1.3 Hepatocellular hypertrophy, increased liver weights and enlarged liver

Increased liver weight, enlarged liver and hepatocellular hypertrophy were observed over time, and the reversibility of these effects was also examined. After 3 days, enlarged livers and increased absolute and relative liver weights were observed in males and females at 1500 ppm and higher. By 14 days, these effects (with the exception of enlarged liver) were present in both sexes at and above 250 ppm. Hepatocellular hypertrophy was evident by day 7 at and above 250 ppm in males and at 3000 ppm in females (Buesen et al., 2010b), but the dose–response relationship for this effect was not as robust as in a second study, which also examined reversibility (Buesen et al., 2009e). In this study, a clear dose-related increase in hepatocellular hypertrophy was observed in males at and above 250 ppm and females at and above 1500 ppm, with increases in absolute and relative liver

weights in both sexes at and above 250 ppm after 2 weeks of fluxapyroxad administration. In a 4-week recovery group, hepatocellular hypertrophy was not observed, and absolute and relative liver weights remained increased in males only.

A2.1.4 Liver tumours

Adenomas were observed at lower doses in male rats (≥ 1500 ppm) compared with carcinomas (3000 ppm). These tumours were not observed until 2 years. At 2 years, three male rats had both adenomas and carcinomas. Females, which were not as sensitive as males to the tumorigenic effects of fluxapyroxad, did not demonstrate a treatment-related increase in carcinomas and demonstrated adenomas only at 3000 ppm. Together, these data indicated a dose- and time-related progression of adenomas to carcinomas, for which males are more sensitive.

A2.2 Dose–response relationship

The dose–response relationship for the key events leading up to (and including) liver tumour formation is summarized in Table A-1. Males were clearly the more sensitive sex, with adenoma formation at and above 1500 ppm. At 3000 ppm, both adenomas and carcinomas developed in males. In females, adenomas were found only at 3000 ppm, and there were no carcinomas. Several of the underlying key events (liver enzyme induction, cell proliferation, hepatocellular hypertrophy, increased liver weight, enlarged liver) occurred at doses lower than those at which tumour formation occurred (≥ 250 ppm).

Table A-1. Dose–response association of key events of liver tumorigenesis

	Males					Females				
	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm
Liver enzyme induction			X	X	X			X	X	X
Cell proliferation in liver				X	X			X	X	X
Hepatocellular hypertrophy			X	X	X				X	X
Increased liver weight			X	X	X			X	X	X
Enlarged liver				X	X				X	X
Non-neoplastic changes in liver (cysts, macro/micro foci)				X	X				X	X
Adenoma				X	X					X
Carcinoma					X					
Adenoma/carcinoma					X					
Adenoma and carcinoma in same animal					X					

A2.3 Temporal association

The temporal associations for the key events leading up to (and including) liver tumour formation are summarized in Table A-2. Changes in the liver (enzyme induction, cell proliferation, hepatocellular hypertrophy, increased liver weight, enlarged liver) occurred within days. Many of these effects (hepatocellular hypertrophy, increased liver weight, enlarged liver) persisted and were observed in conjunction with tumours. Cell proliferation, however, appeared to be an early event (within 90 days).

Table A-2. Temporal association of key events of liver tumorigenesis

	Males					Females				
	≤ 7 days	≤ 14 days	≤ 28 days	≤ 1 year	≤ 2 years	≤ 7 days	≤ 14 days	≤ 28 days	≤ 1 year	≤ 2 years
Liver enzyme induction	X	X				X	X			
Cell proliferation in liver	X	X		Low at 91 days		X	X	X	Low at 91 days	
Hepatocellular hypertrophy	X	X	X	X	X	X	X	X	X	X
Increased liver weight	X	X	X	X	X	X	X	X	X	X
Enlarged liver	X	X	X	X	X	X	X	X	X	X
Adenomas					X					X
Carinomas					X					
Adenoma/ carcinoma					X					
Adenoma and carcinoma in same animal					X					

A2.4 Strength, consistency and specificity

The effects underlying the key events in this MOA were robust, and many were replicated in different studies, supporting strength and consistency. The liver zone of origin for tumours was not known, and the zones of enzyme induction were not confirmed. However, CYPs are known to be highly expressed in the centrilobular regions (zone 3). Cell proliferation occurred in all zones of the liver, but was highest in zone 3. Also, hepatocellular hypertrophy occurred in the centrilobular region (zone 3). Together, the key events indicated high specificity for tumorigenesis in the liver.

A2.5 Alternative MOAs

- Genotoxicity leading to tumours—There is no evidence of genotoxicity in the fluxapyroxad database. Therefore, any genotoxic and mutagenic MOA would have to be via a reactive oxygen species (ROS)–mediated mechanism associated with direct ROS generation by fluxapyroxad or from inflammation. There is no evidence that fluxapyroxad directly generates ROS, and there is no evidence of inflammatory effects in the liver.
- Activation of peroxisome proliferator-activated receptor alpha (PPARα) leading to tumours—There was no microscopic evidence of peroxisome proliferation.
- Acute cellular toxicity leading to cell death and compensatory cell proliferation—Cell proliferation was observed within 3 days, and so cell death would have to occur earlier for this MOA to be operative. Histopathological examination of the liver was performed on day 1 of oral administration, and no evidence of liver necrosis, inflammation or other signs of acute injury was reported.

A2.6 Conclusion on the postulated mode of action in animals

The overall weight of evidence supported a non-genotoxic mitogenic MOA for fluxapyroxad. Fluxapyorad induced PROD and BROD in the liver, a sharp increase in cell proliferation within 3–7 days and hepatocellular hypertrophy. Over prolonged exposure, hepatocellular adenomas and carcinomas developed. Alternative MOAs were excluded.

A2.7 Human relevance

The human relevance of liver tumours in rats via the proposed MOA is equivocal. PB was used as a positive control in some of the thyroid mechanistic studies (e.g. ^{125}I uptake and perchlorate discharge). These thyroid effects are influenced by liver enzyme regulation, and the results were similar to those of fluxapyroxad. PB is also known to work via a mitogenic MOA, with key events similar to the ones proposed for fluxapyroxad. The relevance of tumours that occur via this MOA to humans is equivocal, however, as no clear relationship between PB exposure and hepatotumorigenesis has been established.

A3. Postulated mode of action: thyroid

Administration of fluxapyroxad was associated with a statistically significant increase in the incidence of combined follicular cell adenomas and carcinomas (3, 2, 5, 9 and 11 at 0, 50, 250, 1500 and 3000 ppm, respectively) in the thyroid in male rats at the highest dose tested, with a non-statistically significant increase at 1500 ppm. The incidence at all doses was within the historical control range (4–30%), although the incidence at and above 1500 ppm exceeded the mean (15%). In mice, no treatment-related thyroid tumours were observed in either sex at any dose. The proposed MOA for thyroid follicular cell adenomas and carcinomas is that fluxapyroxad induces liver enzymes leading to increased excretion of thyroid hormone and compensatory changes in thyroid hormone production. These compensatory changes then lead to increased hypertrophy and hyperplasia in the thyroid, which, over time, progresses to tumours.

A3.1 Key events in the mode of action: thyroid

A3.1.1 Liver enzyme induction

Fluxapyroxad administration for 14 days caused increases in total liver CYP and induction of PROD/BROD, which are associated with CYP2B in particular. This occurred at doses of 250 ppm and above (Buesen et al., 2009e).

Also after 14 days, increased thyroxine uridine diphosphate–glucuronosyl transferase (T_4-UDP-GT) is observed in males and females at and above 1500 ppm. This increase is 57–59% in males and 176–183% in females. This was accompanied by increases in phase I liver enzymes in both sexes at and above 250 ppm. The increases in T_4-UDP-GT were reversible 4 weeks after cessation of oral exposure (Buesen et al., 2009e).

A3.1.2 Increased excretion of thyroid hormones and compensatory changes in thyroid hormone levels

TSH levels were increased approximately 70% and T_4 levels were decreased approximately 15–20% in males by 14 days of oral administration of fluxapyroxad at 3000 ppm (Buesen et al., 2009f). Increases in TSH by 14 days at 3000 ppm in males were reversible 4 weeks after cessation of treatment (Buesen et al., 2009e). In the 90-day study, T_3 levels were increased in a dose-responsive manner at and above 100 ppm (11–51%).

Also after 14 days, increased T_4-UDP-GT was observed in males and females at and above 1500 ppm. This increase was 57–59% in males and 176–183% in females. This was accompanied by increases in phase I liver enzymes in both sexes at and above 250 ppm. The increases in T_4-UDP-GT were reversible 4 weeks after cessation of oral exposure (Buesen et al., 2009e).

A perchlorate discharge assay showed that after 14 days of exposure to 3000 ppm fluxapyroxad, pulsing with ^{125}I and discharge with perchlorate, a significant amount of ^{125}I remained, which was similar to the findings with a PB control. This indicated that the effects of fluxapyroxad on the thyroid were indirect, by interfering with hormone homeostasis, and not direct, by interfering with thyroid hormone biosynthesis, as observed for the PTU control (Buesen et al., 2009g).

A3.1.3 Thyroid hypertrophy/hyperplasia/increased thyroid weights

Histopathology shows a dose-related increase in thyroid follicular cell hypertrophy/ hyperplasia and altered colloid in males at and above 250 ppm after 14 days of exposure. Females were less sensitive, showing thyroid follicular cell hypertrophy/hyperplasia at 3000 ppm only. These histopathological effects are decreased in incidence and severity or completely reversed 4 weeks following cessation of oral exposure (Buesen et al., 2009e).

Increased thyroid weights were observed throughout the toxicity database for fluxapyroxad. In addition, significant increases in absolute and relative thyroid weights were observed in males as early as 3 days at and above 50 ppm (Buesen et al., 2010b) and in females as early as 14 days at 3000 ppm (Buesen et al., 2009e). However, an attempt to demonstrate reversibility of thyroid weights at day 28 actually showed no change in thyroid weights in treated males.

An increase in thyroid follicular cell adenomas and combined adenomas/carcinomas was observed only in males and only at the high dose, with one high-dose rat having developed both adenomas and carcinomas (Table A-3).

Table A-3. Incidence of thyroid tumours in male rats following 2 years of treatment

	Incidence of thyroid gland tumours				
	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm
No. examined	*50*	*50*	*50*	*50*	*50*
Adenoma, follicular cell	3	2	4	8	9
Carcinoma, follicular cell			1	1	3
Combined follicular cell tumours	3	2	5	9	11*[a]

From Buesen et al. (2009b)

* $P \leq 0.05$ (comparison of all dose groups with the control group using Fisher's exact test [one-sided] for the hypothesis of equal proportions)

[a] One animal with adenoma *and* carcinoma.

A3.2 Dose–response relationship

The dose–response relationship for the key events leading up to (and including) thyroid tumour formation are summarized in Table A-4. While males and females were equally sensitive to liver enzyme induction at and above 250 ppm and increased excretion of thyroid hormone at and above 1500 ppm, males are more sensitive to compensatory changes in thyroid hormone levels. These compensatory changes do not occur until 3000 ppm and were absent in females. The increase in thyroid follicular cell hypertrophy/hyperplasia occurred at lower doses than changes in thyroid hormone levels in both sexes. The reason for this is unclear (perhaps histopathology was a more sensitive end-point than blood levels of hormones in this case). In any case, thyroid follicular cell hypertrophy/hyperplasia occurred at doses much lower than those that cause increased adenomas and adenomas/carcinomas in males.

A3.3 Temporal association

The temporal associations for the key events leading up to (and including) thyroid follicular cell adenomas and adenomas/carcinomas are summarized in Table A-5. The earliest events (within weeks) are liver enzyme induction, increased excretion of thyroid hormones and changes in thyroid hormone levels. Thyroid follicular cell hypertrophy and hyperplasia are also observed by 2 weeks and persist for 1 and 2 years. Adenomas and adenomas/carcinoma increase for males only.

Table A-4. Dose–response association of key events of thyroid follicular cell adenomas/carcinomas

	Males					Females				
	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm	0 ppm	50 ppm	250 ppm	1500 ppm	3000 ppm
Liver enzyme induction			X	X	X			X	X	X
Increased excretion of T_4-UDP-GT				X	X				X	X
Changes in thyroid hormone levels (↑TSH, ↓T_4)					X					
Thyroid follicular cell hypertrophy/hyperplasia			X	X	X					X
Adenoma					X					
Adenoma/carcinoma					X					

T_4, thyroxine; T_4-UDP-GT, thyroxine uridine diphosphate–glucuronosyl transferase; TSH, thyroid stimulating hormone

Table A-5. Temporal association of key events of thyroid follicular cell adenomas/carcinomas

	Males					Females				
	≤ 7 days	≤ 14 days	≤ 28 days	≤ 1 year	≤ 2 years	≤ 7 days	≤ 14 days	≤ 28 days	≤ 1 year	≤ 2 years
Liver enzyme induction	X	X				X	X			
Increased excretion of T_4-UDP-GT		X					X			
Changes in thyroid hormone levels (↑TSH, ↓T_4)		X								
Thyroid follicular cell hypertrophy/hyperplasia		X	X	X	X		X	X	X	X
Adenoma					X					
Adenoma/carcinoma					X					

T_4, thyroxine; T_4-UDP-GT, thyroxine uridine diphosphate–glucuronosyl transferase; TSH, thyroid stimulating hormone

A3.4 Strength, consistency and specificity

The effects underlying the key events in this MOA are statistically robust, and many are replicated in different studies, which support strength and consistency. The studies underlying the key events in the MOA were conducted using thyroid tissue or plasma from orally dosed animals, and the thyroid hormones measured are specific to the thyroid. Although changes in TSH levels were not observed following 28 days of treatment at doses lower than those resulting in hypertrophy or hyperplasia, enzyme induction was observed at the same dose. Decreased T_4 levels were not observed at doses below those resulting in hypertrophy or hyperplasia, and increased T_3 levels were observed at 90 days at lower doses. Therefore, this MOA is not considered to have adequate strength, consistency or specificity.

A3.5 Alternative MOAs

An alternative MOA is genotoxicity leading to thyroid tumours. There is no evidence of genotoxicity in the fluxapyroxad database. Therefore, any genotoxic MOA would have to be via an

ROS-mediated mechanism associated with direct ROS generation by fluxapyroxad or from inflammation. There is no evidence that fluxapyroxad directly generates ROS, and there is no evidence of inflammatory effects in the thyroid.

The other alternative MOA is a direct effect on thyroid hormone biosynthesis. A perchlorate discharge study did not demonstrate an impact of fluxapyroxad on ^{125}I uptake by the thyroid.

A3.6 Conclusion on the postulated mode of action in animals

The overall weight of evidence did not support a mitogenic MOA for fluxapyroxad through indirect effects on the thyroid. Fluxapyroxad increased T_4-UDP-GT in the liver, but doses were not concordant with increased excretion of T_4 from the blood. Decreased serum T_4 levels continuously stimulate TSH production, resulting in follicular cell hyperplasia, leading to the development of follicular cell tumours. Rodents are sensitive to the induction of thyroid follicular cell tumours, first because of easy induction of T_4-UDP-GT in rodent species and second because of rapid T_4 metabolism in the absence of thyroxine binding globulin in the serum of rodents. Direct effects of fluxapyroxad on the thyroid were excluded.

GLUFOSINATE-AMMONIUM

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Explanation

Glufosinate-ammonium is the International Organization for Standardization (ISO)–approved name for ammonium-DL-homoalanin-4-yl(methyl)phosphinate (International Union of Pure and Applied Chemistry), with Chemical Abstracts Service number 77182-82-2. Glufosinate-ammonium is used as a non-selective herbicide for total vegetation control and as a desiccant to aid in crop harvesting. Glufosinate-ammonium, a racemic mixture of the D- and L-isomers, is a phosphinic acid analogue of glutamic acid. Its herbicidal action is related to the inhibition of glutamine synthetase, an enzyme that plays an important role in ammonia detoxification, amino acid metabolism and protein and nucleotide biosynthesis in plants.

Glufosinate-ammonium was previously evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 1991 and 1999. In 1999, the Meeting established a group acceptable daily intake (ADI) of 0–0.02 mg/kg body weight (bw) for glufosinate-ammonium, 3-[hydroxy(methyl) phosphinoyl]propionic acid (MPP) and *N*-acetyl-glufosinate (NAG) (alone or in combination) on the basis of a no-observed-adverse-effect level (NOAEL) of 2 mg/kg bw per day in a long-term study in rats given technical-grade glufosinate-ammonium.

Glufosinate-ammonium was re-evaluated by the present Meeting as part of the periodic review programme at the request of the Codex Committee on Pesticide Residues. The present Meeting evaluated studies on glufosinate as well as studies on 3-methylphosphinico-propionic acid (= MPP), NAG and 2-methylphosphinico-acetic acid (MPA), three metabolites that are found in plants, soil and livestock. Both the new data and previously submitted studies were considered by the present Meeting.

All critical studies complied with good laboratory practice (GLP).

Evaluation for acceptable daily intake

A. GLUFOSINATE-AMMONIUM

The structure of glufosinate-ammonium is shown in Figure 1.

Figure 1. Structure of glufosinate-ammonium

D,L-glufosinate-ammonium

1. Biochemical aspects

1.1 Absorption, distribution and excretion

Rats

[^{14}C]Glufosinate-ammonium (radiochemical purity 97–98%), labelled at the 2 position, was administered either intravenously or orally by gavage at a single dose of about 2 mg/kg bw to groups of 10 male and 10 female SPF Wistar rats. The vehicle was polyethylene glycol 400 for intravenous administration or a 2% starch suspension for oral administration. Radioactivity was determined in blood samples taken from the retrobulbar venous plexus at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 24, 32, 48, 72, 96, 120, 144 and 168 hours post-dosing for all animals (five of each sex). For animals dosed intravenously, an additional sample was taken at 0.083 hour. Radioactivity in the expired air was measured in two animals 24 hours after administration. Furthermore, radioactivity was measured on a daily basis in faeces and urine. At day 7 after dosing, the orally dosed rats were killed, and levels of radioactivity in spleen, kidneys, liver, gonads, heart, lung, skeletal muscles, subcutaneous and retroperitoneal fat, brain, bones, blood and plasma were determined.

Total recovery of radioactivity was 95–96%. During the first 24 hours after oral dosing, no radioactivity was detected in the air. The blood levels after oral administration were low and measurable only in males up to 3 hours and in females up to 8 hours. Maximum concentrations (0.008 µg/ml in males, 0.029 µg/ml in females) were reached 0.5–1 hour after administration. The half-life in blood was 3.7 hours. After oral administration, 89% and 81% of the radiolabel were excreted in faeces in males and females, respectively, whereas 7% and 14% were excreted in urine of males and females, respectively. Radiolabel (< 0.2 µg equivalent [Eq] per gram tissue) was found only in kidneys, testes and liver, and at low levels. After intravenous administration, 85% and 93% of the dose were excreted in urine of males and females, respectively, whereas 18% and 8% were excreted in faeces of males and females, respectively. Plasma levels were 4.5 and 5 µg Eq/ml in males and females, respectively. After intravenous administration, renal elimination was biphasic, with half-lives of 7 and 52 hours in males and 8 and 64 hours in females (Kellner & Eckert, 1983).

[^{14}C]Glufosinate-ammonium (radiochemical purity > 99%), labelled at the 3 and 4 positions of the aminobutyric acid group and dissolved in a saline solution, was intravenously administered into the tail vein of 10 male Wistar rats at a single dose of approximately 2.3 mg/kg bw. Groups of five rats were killed 2 or 24 hours after dosing. Urine and faeces of the second group were collected separately. Each animal was exsanguinated, and kidney, liver, brain and blood were sampled

individually. All samples were radioassayed by liquid scintillation counting (LSC). Metabolite characterization was performed in faeces, urine, liver, kidney and brain and is described in section 1.2 in the evaluation of the study of Lauck-Birkel & Strunk (1999a). Statements of adherence to quality assurance (QA) and GLP were included.

Twenty-four hours after intravenous dosing, 84.5% of the administered dose was recovered, predominantly in urine (80.5% of the dose, including 2.7% in cage wash), with 2.4% excreted in the faeces. Total radioactivity levels in kidneys, liver, brain and blood amounted to 7.3% and 1.7% of the administered dose at 2 and 24 hours after administration, respectively (Maas & Braun, 1999a).

^{14}C-labelled glufosinate-ammonium (radiochemical purity 98%) was administered by gavage to five male and five female Wistar rats via stomach tube at a dose of 31.5–31.6 mg/kg bw. Urine and faeces were collected at 0–2, 2–4, 4–8 and 8–24 hours and at daily intervals up to 7 days thereafter. At day 7, the rats were killed, and blood, spleen, kidneys, liver, gonads, heart, lung, skeletal muscle, subcutaneous and renal fat, brain and bones were sampled. All excreta and organ/tissue samples were radioassayed by LSC. Statements of adherence to QA and GLP were included.

In total, 95–96% of the administered radioactivity was recovered. Faecal excretion accounted for 88% and 84% of the administered radiolabel in males and females, respectively, whereas 7% and 11% were recovered from urine in males and females, respectively. More than 70% was excreted in the 0- to 24-hour collection interval in both sexes. The renal and faecal excretion patterns suggested a biphasic excretion rate. The half-lives of the renal excretion were 6–7 and 36 hours for both sexes. The faecal excretion half-lives were 5–6 and 38 hours. After 7 days, 0.06–0.07% of the administered dose was detected in organs and tissues of the rats (Kellner & Eckert, 1985a).

^{14}C-labelled glufosinate-ammonium (radiochemical purity 98%) was orally administered to groups of three male and three female Wistar rats by gavage at 800 mg/kg bw. Urine and faeces were collected 0–6 and 0–24 hours after dosing. At the end of the collection period, the rats were killed, and kidneys, liver, spleen and brain were dissected. The excreta and organ samples were radioassayed by LSC and analysed by radio–high-performance liquid chromatography (radio-HPLC) and co-elution with reference standards. Metabolite characterization is described in section 1.2. Statements of adherence to QA and GLP were included.

Twenty-four hours after dosing, 21–24% of the dose was excreted with the faeces, and approximately 4% with the urine. The excretion pattern was independent of sex. In total, less than 0.20% of the radioactivity administered was present in the selected organs and tissues. At 6 and 24 hours after dosing, the highest levels of radiolabel were detected in kidneys (15–77 µg Eq/g). Lower radioactivity levels were found in liver (6–16 µg Eq/g) and spleen (9–18 µg Eq/g), whereas still lower levels were found in the brain (0.5–1.9 µg Eq/g) (Schwalbe-Fehlet al., 1985).

Following administration of ^{14}C-labelled glufosinate-ammonium (radiochemical purity 98%) by gavage at a dose of 800 mg/kg bw to three male and three female rats, blood samples (0.1 ml) were taken from the retrobulbar venous plexus and radioassayed at 0.25, 0.5, 1, 2, 4, 6, 7.5 and 24 hours after administration, and radioactivity levels were measured. Statements of adherence to QA and GLP were included.

Blood levels reached more than 70% of the maxima at the first sampling interval 0.25 hour after dosing in the animals of both sexes, indicating a rapid absorption. The maximum blood levels (3.18 and 3.32 µg Eq/ml in males and females, respectively) were reached 0.5–1.0 hour after dosing. The elimination of the residues from the blood occurred in an initial rapid (half-life 4–5 hours) and a subsequent slower phase. No sex differences were observed (Kellner & Eckert, 1985b).

A group of five male and five female Wistar rats (WISKf (SPF 71)) were gavaged with [^{14}C]glufosinate-ammonium (radiochemical purity 98%; labelled at the 3 and 4 positions of the aminobutyric acid group) at a dose of 2.5 mg/kg bw for males and 2.9 mg/kg bw for females. The test substance was dissolved in water. Faeces, urine and cage wash were collected once daily over 4 days, and each group of samples was pooled for each sex. At 96 hours, the animals were killed, liver, kidney, fat, brain, spleen and blood were sampled and each group of samples was pooled for each sex. Radioactivity levels in urine, faeces and organ samples were measured. Metabolite profiles in urine and faeces are described under section 1.2. Statements of adherence to QA and GLP were included.

Total recoveries of radioactivity were 98–100%. Within 24 hours, 88% and 81% were excreted in faeces of males and females, respectively, and 6% was excreted in urine of both sexes. The highest residues were detected in kidneys (0.098 and 0.217 mg Eq/kg in males and females, respectively) and liver (0.038 and 0.027 mg Eq/kg in males and females, respectively). Fat of male rats contained residue levels of 0.038 mg Eq/kg. Other residue levels were less than or equal to 0.014 mg Eq/kg (Wink, 1986a).

^{14}C-labelled glufosinate-ammonium (radiochemical purity > 99%) was orally administered to 10 male and female Wistar rats via gavage after 14 doses of non-labelled glufosinate-ammonium (purity 98.6%) at a dose of 2 mg/kg bw per day. Urine and faeces were collected on a daily basis and each pooled per sex and collection interval. All rats were sacrificed 96 hours after the radioactive dose, and liver, kidney, fat, brain, spleen and blood were sampled. All samples were radioassayed. Statements of adherence to QA and GLP were included.

After 96 hours, the total excretion in faeces was 83.0% and 81% and in urine was 5% and 6% of the dose in male and female rats, respectively. The majority was excreted within the first 24 hours. At 96 hours after dosing, the kidneys and livers of males contained 0.276 and 0.055 µg Eq/g, whereas in females, 0.108 and 0.033 µg Eq/g were found. Blood, brain and spleen contained maximally 0.003 µg Eq/g in both sexes. The route and rate of excretion after repeated administration and the residue level in selected organs and tissues were similar to those after a single oral dose of about 2 mg/kg bw (see above) (Wink, 1986b).

[^{14}C]Glufosinate-ammonium (radiochemical purity > 98%; labelled at the 3 and 4 positions of the aminobutyric acid group) was administered to groups of five male and five female Wistar rats via gavage at 500 mg/kg bw. Urine and faeces were collected on a daily basis. The groups were killed 24 or 96 hours after administration, at which time samples of spleen, kidneys, liver, brain, blood and plasma were collected. Extra single male and female rats also received an oral dose of 500 mg/kg bw, but were killed 2 and 6 hours after dosing. Metabolite characterization is described in section 1.2. Statements of adherence to QA and GLP were included.

Most (87–96%) of the dose was recovered in urine (8–11%) and faeces (75–89%) after 96 hours. Tissue radioactivity levels were highest 2 hours after dosing in kidney (76–81 µg Eq/g), spleen (12–41 µg Eq/g), blood (0.8–1 µg Eq/g) and plasma (3 µg Eq/g) and 6 hours after dosing in liver (12–18 µg Eq/g) and brain (0.6–1.1 µg Eq/g). No sex differences were observed (Lauck-Birkel, 1995a).

[^{14}C]Glufosinate-ammonium (radiochemical purity > 99%; labelled at the 3 and 4 positions of the aminobutyric acid group) dissolved in a saline solution was administered by gavage to groups of five male Wistar rats at a single dose of 20 mg/kg bw. The groups were killed 1, 6 or 24 hours after dosing. Urine and faeces of the 24-hour group were collected separately. Each animal was exsanguinated, and kidney, liver, brain, blood and plasma were sampled. Metabolite characterization performed in faeces, urine, liver and kidney is described in section 1.2 in the evaluation of the study of Lauck-Birkel & Strunk (1999b). Statements of adherence to QA and GLP were included.

Twenty-four hours after dosing, 92% of the dose was excreted with the faeces and 4.9% with the urine, and 0.2% of the dose was detected in the dissected organs and blood. Peak levels of radioactivity were detected in kidney (3.4 µg Eq/g) and blood (0.2 µg Eq/g) 1 hour after dosing, in liver (0.72 µg Eq/g) 6 hours after dosing and in brain (0.034 µg Eq/g) 24 hours after dosing (Maas & Braun, 1999b).

^{14}C-labelled glufosinate-ammonium (radiochemical purity > 97%) was orally administered to five male and five female Wistar rats via gavage after 14 doses of non-labelled glufosinate-ammonium (purity not reported) at a dose rate of 2 mg/kg bw per day. One male and one female rat served as control. Urine and faeces, collected at 2, 4, 8 and 24 hours and on a daily basis thereafter, were radioassayed. All animals were sacrificed 7 days after administration of the radioactive dose, and the organs and tissues were dissected and radioassayed. Statements of adherence to QA and GLP were included.

About 89–91% and 8–9% of the radiolabel were excreted in faeces and urine, respectively. About 90% of the total radioactivity was excreted within 24 hours. Both the renal excretion and the faecal excretion were biphasic. The half-lives for renal excretion were calculated to be 5–6 hours for the first phase and approximately 1.5 days for the second phase. For faecal excretion, the half-lives were 5 hours for the first phase and 1.3–2 days for the second phase. No sex-related differences were observed. In comparison with single-dose studies (Wink, 1986a; Stumpf, 1993a), the repeated dosing did not change the excretion pattern. Seven days after dosing, radioactive residues in the kidneys, testes, liver and carcass of males amounted to 0.138, 0.078, 0.028 and 0.029 µg Eq/g, respectively. At this time in females, radioactive residues in the kidneys, liver and spleen accounted for 0.047, 0.023 and 0.008 µg Eq/g, respectively. Mean levels of radioactivity in other organs and tissues were below the limit of detection (Kellner & Eckert, 1985c).

Glufosinate-ammonium was administered orally by gavage to groups of three female Wistar rats (WISKf, SPF 71) at about 12 or 109 mg/kg bw once a day for 10 days; on days 1, 8, 9 and 10, the animals received a dose of [^{14}C]glufosinate-ammonium (purity 99%), whereas on days 2–7, they were given non-labelled glufosinate-ammonium (purity 99.5%) at the same two doses. Radioactivity in blood was determined on days 1, 8, 9 and 10 at 1, 2, 4, 6, 8 and 24 hours after dosing. Statements of adherence to QA and GLP were included.

Maximum blood levels were observed 1–2 hours after oral administration of [^{14}C]glufosinate-ammonium, ranging from 0.11 to 0.25 µg Eq/ml at 12 mg/kg bw and from 1.25 to 1.65 µg Eq/ml at the 109 mg/kg bw dose. Blood levels increased slightly after two successive radioactive doses, but did not further increase after three successive radioactive doses at both dose levels. The elimination half-lives in the blood were 2.3–5.3 hours after both doses (Kellner & Eckert, 1986).

Five male and five female Wistar rats received seven daily doses of [^{14}C]glufosinate-ammonium (radiochemical purity ≥ 98%) at 2 mg/kg bw per day by gavage. Urine and faeces were collected on a daily basis until 4 days after the last dose and each pooled per sampling interval and sex. The animals were killed and dissected 96 hours after the last dose, and a wide range of organs and tissues was sampled and radioassayed. Statements of adherence to QA and GLP were included.

At 96 hours after the last dose of radiolabelled glufosinate-ammonium, faecal excretion was 87.4% and 88.2% of the cumulative dose in males and females, respectively, and urinary excretion was 6.5% and 5.9% of the cumulative dose in males and females, respectively (see Table 1). At this time, less than about 0.3% of the cumulative dose remained in the dissected organs and tissues of both sexes. The excretion patterns after multiple doses in this study were similar to those of single-dose studies (see above) (Gutierrez, 2002).

Table 1. Cumulative excretion of radiolabel in male and female rats receiving seven oral doses of ^{14}C-labelled glufosinate-ammonium at 2 mg/kg bw per day

Number of oral doses	Collection period (h)	% of administered radioactivity			
		Males[a]		Females[a]	
		Urine	Faeces	Urine	Faeces
1	0–6	3.08	—	2.19	—
	0–24	5.76	82.19	5.19	82.61
2	0–48	5.92	85.65	4.93	84.24
3	0–72	5.60	88.52	4.94	87.32
4	0–96	5.72	88.44	5.34	88.26
5	0–120	6.15	87.76	5.45	86.64
6	0–144	6.32	86.46	5.67	84.93
7	0–168	6.29	86.80	5.70	87.48
End of administration					
	0–192	6.42	87.37	5.83	88.15
	0–216	6.47	87.40	5.88	88.20
	0–240	6.50	87.40	5.90	88.21

From Gutierrez (2002)
[a] Mean of five animals of each sex.

Groups of three pregnant female Wistar rats at gestation day (GD) 6, 14 or 18 were given a single intravenous injection of ^{14}C-labelled glufosinate-ammonium (purity > 98%) at 2.4 mg/kg bw. Quantitative whole-body autoradiography was performed on one dam from each group 5 minutes, 30 minutes or 1 hour after administration. Whole-body sections from dams, uteri and fetuses were prepared separately. Statements of adherence to QA and GLP were included.

A similar distribution pattern was observed in all stages of pregnancy. At 5 minutes after administration, radioactivity was distributed ubiquitously in the body. The highest concentrations were found in the kidney, lacrimal gland and thymus. Up to 1 hour after administration, the concentration in most of the analysed regions decreased to approximately 10% of the initial values. In kidney, adrenal gland, lacrimal gland, salivary gland, pancreas, thyroid and thymus, the concentration was unchanged or increased slightly.

On GD 6, owing to the small size of the placenta and fetuses, no fetuses could be observed or analysed in the whole-body sections or in the separately prepared uterus. On GD 14 and GD 18, the distribution of the radioactivity was similar. At 5 minutes after administration, the concentration in the blood was about 2 times higher than in the placenta and 20–40 times higher than in the fetus, whereas at 30 minutes after dosing, the concentrations in blood and placenta were similar, but 12–13 times higher than in the fetus. The concentrations in the fetuses were the lowest of all organs and tissues of the pregnant rats, with the exception of the central nervous system. At 1 hour after administration, the radioactivity concentrations decreased considerably in the maternal blood, placenta and fetus.

The distribution of the compound in the body was ubiquitous, with poor penetration of the placental and blood–brain barrier (Maas & Zimmer, 2000).

Rabbits

[^{14}C]Glufosinate-ammonium (radiochemical purity > 99.7%) was administered by gavage as a single oral dose to three female New Zealand White rabbits at 6 mg/kg bw. Urine and faeces were collected on a daily basis for a total period of 10 days. The excreta samples were radioassayed and analysed by radio-HPLC. Statements of adherence to QA and GLP were included.

Total recovery of administered radioactivity was 97.9%. Faecal excretion and urinary excretion were 70.2% and 27.7%, respectively. The cumulative excretion data (Table 2) indicate that excretion in female rabbits was relatively slow compared with that of rats (see above) and dogs (see below) (Koester, 2004).

Table 2. Cumulative excretion of radioactive residues by female rabbits following a single oral dose of [^{14}C]glufosinate-ammonium of 6 mg/kg bw

Time after dosing (h)	Cumulative % of administered radioactivity		
	Urine	Faeces	Urine + faeces
24	1.11	13.88	14.99
48	9.88	25.26	35.14
72	15.57	34.60	50.17
96	19.61	45.01	64.62
120	22.63	53.26	75.89
144	24.52	60.12	84.64
168	25.72	64.23	89.95
192	26.65	66.59	93.24
216	27.25	68.78	96.03
240	27.67	70.24	97.91

From Koester (2004)

Dogs

[^{14}C]Glufosinate-ammonium (radiochemical purity > 98%) was orally administered by gelatine capsule to two male and two female Beagle dogs at a dose of 8 mg/kg bw. Excreta were collected at 6 and 24 hours. Blood was sampled at 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 hours. One male and one female dog were killed 6 hours after dosing, whereas the other two dogs were killed 24 hours after dosing. Levels of radioactivity were determined in heart, liver, kidneys, several parts of the brain and spinal cord. Statements of adherence to QA and GLP were included.

Peak blood levels of radioactivity were detected 2–4 hours after dosing. Radiolabel was almost completely excreted in faeces (82–83% of the dose) and urine (9–10% of the dose) within 24 hours of dosing. In males, the highest residue levels were found in the kidneys (1.2–1.6 µg Eq/g) and liver (0.4–1.3 µg Eq/g) at 6–24 hours after dosing. In females, the highest residue levels were found in the kidneys (2.3–2.4 µg Eq/g) and liver (0.4–1.2 µg Eq/g) at 6–24 hours after dosing. Radioactivity levels in heart and brain were less than or equal to 0.07 µg Eq/g 6 and 24 hours after dosing. No sex differences in excretion or distribution of radioactivity were observed (Ellgehausen, 1986a).

Groups of male and female dogs (six animals per group) were orally administered glufosinate-ammonium (purity unknown) via gelatine capsules; 18 non-labelled daily doses of 1 or 8 mg/kg bw were followed by 10 daily doses of [^{14}C]glufosinate-ammonium (radiochemical purity > 99.7%) at 1 or 8 mg/kg bw. Urine and faeces were collected at 24 intervals from the 1st day of administration of the radiolabelled glufosinate-ammonium and at 6, 12, 24, 48, 72 and 96 hours after the last dosing. Blood was sampled 1 hour after each radiolabelled dose and 2, 4, 6, 24, 48, 72 and 96 hours after the last dose. One male and one female dog of both dose groups were sacrificed 6 hours after the first administration of [^{14}C]glufosinate-ammonium and 6, 24, 48 and 96 hours after the last (10th) administration of [^{14}C]glufosinate-ammonium. The dogs were exsanguinated and dissected, and samples of heart, liver, kidneys and different parts of the cerebral tissue were radioassayed by LSC. The faeces and organ extracts as well as the urine samples were analysed by radio–thin-layer

chromatography (radio-TLC) together with radiolabelled reference substances. Statements of adherence to QA and GLP were included.

The total renal excretion was 14–17% of the total ^{14}C dose at both dose levels and sexes. The faecal excretion accounted for 79–83.5% of the total [^{14}C]glufosinate-ammonium. In the high-dose group, the maximum blood and plasma levels (0.232 and 0.317 µg Eq/ml in male dogs and 0.260 and 0.351 µg Eq/ml in female dogs, respectively) were reached 6 hours after the last dose. Steady-state levels were reached at days 2–5 of the 10-day administration period. The elimination half-life was 46 hours for the whole blood and 16 hours for the plasma of male dogs. In female dogs, the elimination half-lives from blood and plasma were less than 18 hours. The highest residue levels were found 24–48 hours after the last ^{14}C dose in kidneys (1.111 and 0.500 µg Eq/g at the low dose and 6.437 and 5.140 µg Eq/g at the high dose for male and female dogs, respectively) and liver (0.638 and 0.422 µg Eq/g at the low dose and 3.659 and 3.267 µg Eq/g at the high dose for male and female dogs, respectively). At 1 mg/kg bw, the residue levels in the different regions of the brain and the heart were less than or equal to 0.1 µg Eq/g. At 8 mg/kg bw, the residue levels in the different regions of the brain and the heart were less than or equal to 0.6 µg Eq/g. No significant sex differences were observed. The study showed that glufosinate-ammonium has a low potential for accumulation in dogs (Ellgehausen, 1986b).

1.2 Biotransformation

Rats

A group of five male and five female Wistar rats (WISKf (SPF 71)) received by gavage [^{14}C]glufosinate-ammonium (radiochemical purity 93–95%) labelled at the 3 and 4 positions of the aminobutyric acid group at a dose level of 2.1 mg/kg bw. The test substance was dissolved in saline. Faeces and urine (without cage wash) were collected once daily over 4 days and each pooled for each sex. Metabolite profiles in urine and faeces were investigated by radio-HPLC or radio-TLC. Statements of adherence to QA and GLP were included.

Total recoveries of radioactivity were 101–104%. Levels of radioactivity and residue pattern in urine and faeces, expressed as a percentage of the administered dose, are presented in Table 3.

In urine, parent compound represented about 50% of residue, whereas 4-methylphosphinico-butanoic acid (MPB) and MPP each represented 8–22% of residue. Very low levels of MPA were found in urine. The study shows that following a single oral administration of [^{14}C]glufosinate-ammonium, the radiolabel is rapidly and virtually completely excreted in rats, with the faeces as the main excretion route. The major residue component was the parent compound. No relevant sex differences were observed (Stumpf, 1993a).

A group of three male Wistar (WISKf (SPF 71)) rats received by gavage [^{14}C]glufosinate-ammonium (radiochemical purity > 98%) labelled at the 3 and 4 positions of the aminobutyric acid group at a dose level of 2 mg/kg bw. The test substance was dissolved in saline. Faeces and urine (without cage wash) were collected once daily over 2 days, and samples of each were pooled. Potential microbial degradation of excreted residues during collection was prevented by cooling an addition of aqueous sodium azide. Metabolite profiles in urine and faeces were investigated by radio-HPLC or radio-TLC. Statements of adherence to QA and GLP were included.

Total recoveries of radioactivity were 96–100%. Levels of radioactivity and residue pattern in urine and faeces, expressed as a percentage of the administered dose, are presented in Table 4.

The study shows that following a single oral administration of [^{14}C]glufosinate-ammonium, the radiolabel is rapidly and virtually completely excreted in rats, with the faeces as the main excretion route. The major residue component was the parent compound (Lauck-Birkel, 1996).

Table 3. Residue pattern in urine and faeces of rats after a single oral dose of [^{14}C]glufosinate-ammonium of 2.1 mg/kg bw

	% of the administered dose							
	Males				Females			
	Urine		Faeces		Urine		Faeces	
	0–24 h	0–96 h	0–24 h	0–48 h	0–24 h	0–96 h	0–24 h	0–48 h
Total radioactivity	8.1	9.8	87.6	90.7	7.7	8.6	90.7	95.3
Identified	6.9	8.6	85.3	87.6	6.3	7.2	77.9	81.9
GA	4.1	5.1	73.5	75.3	3.9	4.5	66.0	68.5
MPB	1.5	1.6	< LOD[a]	< LOD	1.7	1.8	< LOD	< LOD
MPP	1.3	1.9	1.3	1.3	0.6	0.8	0.9	0.9
MHB	< LOD	< LOD	3.4	3.6	< LOD	< LOD	2.9	3.3
NAG	< LOD	< LOD	7.1	7.4	< LOD	< LOD	8.1	9.2
MPA	< LOD	< LOD	< LOD	< LOD	0.1	0.1	< LOD	< LOD
Unknown	1.2	1.3	0.6	0.6	1.4	1.5	0.3	0.3

From Stumpf (1993a)
GA, glufosinate-ammonium; LOD, limit of detection; MHB, 2-hydroxy-4-methylphosphinico-butanoic acid; MPA, 2-methylphosphinico-acetic acid; MPB, 4-methylphosphinico-butanoic acid; MPP, 3-methylphosphinico-propionic acid; NAG, *N*-acetyl-glufosinate
[a] Limit of detection is 0.05% of the dose.

Table 4. Residue pattern in urine and faeces of rats after a single oral dose of [^{14}C]glufosinate-ammonium of 2 mg/kg bw

	% of the administered dose			
	Urine		Faeces	
	0–24 h	24–48 h	0–24 h	24–48 h
Total radioactivity	5.3	0.6	90.9	2.8
Identified	5.1	0.6	88.0	2.7
GA	3.92	0.36	75.37	1.82
MPB[a]	0.23	< LOD[b]	0.34	0.09
MPP	0.61	0.17	1.21	0.05
MHB	0.11	0.03	4.07	0.21
NAG	0.08	< LOD	6.98	0.50
MPA	0.10	< LOD	< LOD	< LOD
M1	0.08	0.03	0.19	0.08
Unknown	0.13	< LOD	1.20	0.06

From Lauck-Birkel (1996)
GA, glufosinate-ammonium; LOD, limit of detection; M1, unknown polar metabolite eluted with the void volume of the column; MHB, 2-hydroxy-4-methylphosphinico-butanoic acid; MPA, 2-methylphosphinico-acetic acid; MPB, 4-methylphosphinico-butanoic acid; MPP, 3-methylphosphinico-propionic acid; NAG, *N*-acetyl-glufosinate
[a] MPB was an impurity of the test substance that amounted to 0.68% in the dosing solution.
[b] Limit of detection is 0.02% and 0.05% of the dose for urine and faeces, respectively.

A group of five male and five female Wistar (WISKf (SPF 71)) rats received by gavage [^{14}C]glufosinate-ammonium (radiochemical purity 98%) labelled at the 3 and 4 positions of the aminobutyric acid group at a dose level of 2.5 mg/kg bw for males and 2.9 mg/kg bw for females. The test substance was dissolved in water. Faeces, urine and cage wash were collected once daily over 4 days and each pooled for each sex. Metabolite profiles in urine and faeces were investigated by radio-HPLC or radio-TLC. The toxicokinetics are described under section 1.1. Statements of adherence to QA and GLP were included.

Total recoveries of radioactivity were 98–100%. Only parent compound was detected in urine (6% of the dose) and faeces (79% of the dose) by HPLC analysis. Eleven per cent of radioactivity in faeces was non-extractable (Wink, 1986a).

In the in vivo study of Maas & Braun (1999a) with intravenous dosing of glufosinate ammonium at 2.3 mg/kg bw (see study description in section 1.1), metabolites were analysed in pooled samples of faeces, urine, liver, kidney and brain by radio-HPLC. Blood and plasma were not analysed by radio-HPLC because the radioactivity level was too low. Statements of adherence to QA and GLP were included.

The main residue in all samples was the parent substance glufosinate-ammonium. MPP was also present as a minor metabolite in all samples (see Tables 5 and 6), representing about 10–20% of residue found in liver. In the faeces, additionally minor portions of NAG and 2-hydroxy-4-methylphosphinico-butanoic acid (MHB) could be detected. In 24-hour post-dosing samples, the glufosinate-ammonium/MPP ratio was slightly increased in kidneys but decreased in liver when compared with the corresponding 2-hour post-dosing samples (Lauck-Birkel & Strunk, 1999b; Maas & Braun, 1999a).

Table 5. Residue profile in the 0- to 24-hour excreta of male rats after intravenous administration of approximately 2 mg/kg bw of [^{14}C]glufosinate-ammonium

Compound	% of the administered dose	
	Urine	Faeces
GA	68.1	2.0
MPB	< LOD	< LOD
MPP	9.5	0.05
MHB	< LOD	0.11
NAG	< LOD	0.2
MPA	< LOD	< LOD
Unknown	0.4	< LOD
Identified	77.6	2.4

From Lauck-Birkel & Strunk (1999b); Maas & Braun (1999a)
GA, glufosinate-ammonium; LOD, limit of detection; MHB, 2-hydroxy-4-methylphosphinico-butanoic acid; MPA, 2-methylphosphinico-acetic acid; MPB, 4-methylphosphinico-butanoic acid; MPP, 3-methylphosphinico-propionic acid; NAG, *N*-acetyl-glufosinate

^{14}C-labelled glufosinate-ammonium (radiochemical purity 98%) was orally administered to groups of three male and three female Wistar rats via stomach tube at 800 mg/kg bw. Urine and faeces were collected 0–6 and 0–24 hours after dosing. At the end of the collection period, the rats were killed, and kidneys, liver, spleen and brain were dissected. The excreta and organ samples were radioassayed by LSC and analysed by radio-HPLC and co-elution with reference standards. An additional group of three male and three female rats was used for the investigation of blood kinetics

for 24 hours. The kinetics data are described in section 1.1. Statements of adherence to QA and GLP were included.

Table 6. Residue profile in kidneys, liver and brain of male rats after intravenous administration of approximately 2 mg/kg bw of [^{14}C]glufosinate-ammonium

	2 h post-dosing		24 h post-dosing	
	% of dose	mg Eq/kg	% of dose	mg Eq/kg
Kidneys				
TRR	5.47	15.08	0.52	1.39
Identified	5.47	15.07	0.52	1.40
GA	4.98	13.72	0.49	1.33
MPP	0.49	1.35	0.03	0.08
Liver				
TRR	1.63	0.76	1.15	0.56
Identified	1.62	0.75	1.14	0.56
GA	1.47	0.68	0.93	0.45
MPP	0.16	0.07	0.22	0.10
Unknown	0.01	0.01	0.01	< 0.01
Brain				
TRR	0.02	0.06	0.02	0.04
Identified	0.02	0.06	0.20	0.04
GA	0.02	0.04	0.01	0.03
MPP	< 0.01	< 0.01	< 0.01	0.01
Unknown	< 0.01	< 0.01	—	—

From Lauck-Birkel & Strunk (1999b); Maas & Braun (1999a)
GA, glufosinate-ammonium; MPP, 3-methylphosphinico-propionic acid; TRR, total radioactive residue

The major residue in urine was the parent compound, whereas low levels of the metabolites MPP and MPB were detected. In kidneys and liver, the major residue was also parent compound (73% and 48%, respectively), but MPP (12% and 29%, respectively) and MPB (16% and 23%, respectively) were also found in these organs. In faeces, only parent compound was detected. Because of the very low radioactivity concentrations in the spleen and brain extracts and the high sample background, only parent compound could be identified (Schwalbe-Fehl et al., 1985).

In the study of Maas & Braun (1999b), groups of five male rats were treated by gavage with a single dose of [^{14}C]glufosinate-ammonium at 20 mg/kg bw. The groups were killed 1, 6 or 24 hours after dosing, and samples of urine, faeces, kidney and liver were collected. The present study describes the metabolite characterization by radio-HPLC and co-elution with reference standards of the samples taken in the Maas & Braun (1999b) study. Statements of adherence to QA and GLP were included.

In samples of urine and faeces collected over 0–24 hours, the unchanged parent substance glufosinate-ammonium was the predominant residue component. A minor metabolite in both urine and faeces was identified as MPP (≤ 1%). MPB and MHB were detected in the urine only at trace levels, whereas NAG was detected in the faeces only as a minor metabolite (3% of the dose). In kidneys and liver, the parent substance glufosinate-ammonium was also generally the major residue component at all three sampling intervals. The level of glufosinate-ammonium decreased with time in the kidneys, whereas it slightly increased with time in the liver. MPP peaked at the first sampling

interval in the kidneys, followed by a decrease with time. In the liver, MPP peaked at the second sampling interval (6 hours after administration). MPB was detected at trace levels at all sampling intervals in both organs, with the maximum level at the second sampling interval. NAG could not be detected in the kidney and liver (Lauck-Birkel & Strunk, 1999a).

[^{14}C]Glufosinate-ammonium (radiochemical purity > 98%) labelled at the 3 and 4 positions of the aminobutyric acid group was orally administered to groups of five male and five female Wistar rats by gavage at 500 mg/kg bw. Urine and faeces were collected on a daily basis. The groups were killed 24 or 96 hours after administration, at which time samples of spleen, kidney, liver, brain, blood and plasma were collected. Extra single male and female rats also received an oral dose of 500 mg/kg bw, but were killed 2 and 6 hours after dosing. The samples were analysed for the metabolite profile by radio-HPLC and co-elution with authentic radiolabelled reference standards. Radio-TLC served for confirmation of the HPLC results. Statements of adherence to QA and GLP were included.

Of the radioactivity excreted with urine and faeces (0–96 hours), approximately 82% and 91% of the dose were identified in male and female rats, respectively. The unchanged parent substance glufosinate-ammonium proved to be the predominant residue component in urine and faeces (see Table 7). The metabolite MPP appeared as a minor metabolite, with proportions increasing with collection time in urine and faeces. MPB was found at a constant but low level in all samples of urine and faeces; it was assumed to be an impurity of the test substance. NAG was assumed to be exclusively formed in the intestine by action of the intestinal microflora, as only very small amounts could be detected in the 0- to 24-hour urine. The metabolite MHB was observed only in the faeces at low levels.

Table 7. Excretion profiles in urine and faeces of rats orally administered [^{14}C]glufosinate-ammonium at a dose of approximately 500 mg/kg bw

	% of administered dose							
	Males				Females			
	Urine		Faeces		Urine		Faeces	
	0–24 h	0–96 h	0–24 h	0–96 h	0–24 h	0–96 h	0–24 h	0–96 h
GA	2.52	5.92	37.51	72.11	2.22	4.27	49.30	84.0
MPB	0.19	0.22	0.22	0.29	0.17	0.17	0.11	0.11
MPP	0.46	1.20	0.31	0.58	0.24	0.51	0.31	0.44
MHB	< LOD[a]	< LOD	0.13	0.26	< LOD	< LOD	0.17	0.28
NAG	0.04	0.04	0.35	1.23	0.02	0.02	0.58	1.74
MPA	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Non-anionic unknown M1	0.15	0.19	< LOD	< LOD	0.13	0.20	< LOD	< LOD
Series of unknowns (≤ 5)	0.06	0.12	0.09	0.24	< LOD	< LOD	0.14	0.14
Identified	3.21	7.38	38.52	74.47	2.65	4.97	50.47	86.57

From Lauck-Birkel (1995a)

GA, glufosinate-ammonium; LOD, limit of detection; MHB, 2-hydroxy-4-methylphosphinico-butanoic acid; MPA, 2-methylphosphinico-acetic acid; MPB, 4-methylphosphinico-butanoic acid; MPP, 3-methylphosphinico-propionic acid; NAG, *N*-acetyl-glufosinate

[a] LOD (urine): 0.02% of the administered dose; LOD (faeces): 0.07–0.19% of the administered dose.

A comparison of the metabolite profile in urine and faeces with that observed in a low-dose (2 mg/kg bw) study indicated a very similar residue pattern independently from the dose, with one exception. The proportion of NAG in the faeces was relatively higher after low-dose administration (7–8% of the dose; Lauck-Birkel, 1996), whereas the proportion was only 1–2% of the dose after

high-dose administration (this study). This lower formation rate in the high-dose study suggested some saturation effects in the acetylating microbial enzyme systems (Lauck-Birkel, 1995a).

^{14}C-labelled glufosinate-ammonium (radiochemical purity > 99%) was orally administered to 10 male and 10 female Wistar rats via gavage after 14 doses of non-labelled glufosinate-ammonium (purity 98.6%) at a dose of 2 mg/kg bw per day. Urine and faeces were collected on a daily basis and each pooled per sex and collection interval. All rats were sacrificed 96 hours after the radioactive dose. Metabolic profiles in urine and extracts of the faeces were analysed by radio-HPLC and co-elution of authentic radiolabelled reference standards. Statements of adherence to QA and GLP were included.

Glufosinate-ammonium was the predominant residue component, accounting for 74% of the renally excreted and 80% of the faecally excreted radioactive residues. MPP appeared as a minor metabolite (≤ 0.7% of the dose in urine and ≤ 7.5% of the dose in the faeces of both sexes). A further metabolite, MPB, was found at low levels in both sexes (Wink, 1986b).

Glufosinate-ammonium was administered by gavage to groups of female Wistar (WISKf, SPF 71) rats at about 10 or 100 mg/kg bw once a day for 10 days. On days 1, 8, 9 and 10, the animals received radiolabelled [^{14}C]glufosinate-ammonium (purity 99%), and on days 2–7, non-labelled glufosinate-ammonium (purity 99.6%). Groups of females were killed either 24 hours after the first dose or 24 or 48 hours after the 10th dose. Radioactivity levels in urine, faeces, liver, spleen, kidneys and brain were determined, and the residues in urine and faeces were identified. Statements of adherence to QA and GLP were included.

Following oral administration of [^{14}C]glufosinate-ammonium, excretion was rapid, mainly through faeces (73–103%). Less than 5% was excreted in urine. The excretion pattern was not affected by the repeated dosing. Most (94%) of the excreted radiolabel consisted of parent compound. The metabolites MPP and MPB accounted for 5% and 1%, respectively. Liver, kidney, spleen and brain contained less than 0.1% of the administered radioactivity. No evidence of significant accumulation was observed by repeated dosing (Schwalbe-Fehl, 1986).

Five male and five female Wistar rats received seven daily doses of [^{14}C]glufosinate-ammonium (radiochemical purity ≥ 98%) at 2 mg/kg bw per day by gavage. Urine and faeces were collected on a daily basis until 4 days after the last dose and each pooled per sampling interval and sex. The animals were killed and dissected 96 hours after the last dose. Urine and water extracts of the faeces were profiled by radio-HPLC for the composition of radioactive residues. Statements of adherence to QA and GLP were included.

In urine, at least four different residue components could be detected. The major component, at 2–4.5% of the administered dose, was the parent substance glufosinate-ammonium. Minor metabolites detected were MPP (0.1–0.7% of the dose) and MPB (0–0.2% of the dose) and an unknown, polar peak (0.4–1% of the dose). In the faeces, at least seven different residues were present, with the parent substance as the main component (74–78% of the dose) throughout the study period. The metabolite NAG slightly increased with the number of administrations from 2% to 6% of the administered dose. This metabolite was not detected in urine, suggesting that it is formed only in the intestine or faeces. The metabolites MPB and MPP were observed at levels of about 2–3% of the dose throughout the study period. In addition, low levels of two unknown polar residues (< 1% of the dose) and a non-polar residue (< 0.5% of the dose) were found. No sex differences in the metabolic profile were observed. Furthermore, repeated dosing did not change the metabolite pattern in the urine (Gutierrez, 2002).

Rabbits

[^{14}C]Glufosinate-ammonium (radiochemical purity > 99.7%) was administered by gavage as a single oral dose to three female New Zealand White rabbits at 6 mg/kg bw. Urine and faeces were collected on a daily basis for a total period of 10 days. The excreta samples were radioassayed and analysed by radio-HPLC. Statements of adherence to QA and GLP were included.

HPLC analysis showed that in urine and faeces, mainly glufosinate-ammonium was identified. Two other (unknown) metabolites were also present in urine and faeces. Levels of metabolites were not quantified (Koester, 2004).

Dogs

[^{14}C]Glufosinate-ammonium was orally administered by gelatine capsule to two male and two female Beagle dogs at a dose of 8 mg/kg bw. Excreta were collected at 6 and 24 hours. Blood was sampled at 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 hours. One male and one female dog were killed 6 hours after dosing, whereas the other two dogs were killed 24 hours after dosing. The identity and levels of residues in faeces, urine, plasma, kidneys and liver were determined by radio-TLC. The toxicokinetics of glufosinate-ammonium in dogs is described under section 1.1. Statements of adherence to QA and GLP were included.

Urine contained predominately glufosinate-ammonium (8.0–8.5% of the dose) and low levels of MPP (1.2% of the dose). No sex difference in the metabolite profile in urine was observed. Only the parent compound could be identified in faeces, liver, kidneys and plasma (Ellgehausen, 1986a).

The metabolism of glufosinate-ammonium after repeated doses of 1 or 8 mg/kg bw per day in dogs was examined. The study design is described in section 1.1 (Ellgehausen, 1986b). Samples of heart, liver, kidneys and different parts of the cerebral tissue were radioassayed by LSC. The faeces, urine, liver and kidney samples were analysed by radio-TLC, together with radiolabelled reference substances. Statements of adherence to QA and GLP were included.

Glufosinate-ammonium was the major component in urine and faeces. MPP was observed as a minor metabolite, accounting for less than 1% of the total ^{14}C dose in urine and faeces. In liver and kidneys of the highly dosed dogs, MPP was the predominant residue (31–72% of total residue), except in the liver of the female animals 24 hours after the last dose, in which only glufosinate-ammonium was identified (74% of the total residue). In the other liver and kidney samples, glufosinate-ammonium amounted to 11–40% of the total residue. The excretion pattern and the residue levels in selected organs were comparable to those observed in rats dosed with [^{14}C]glufosinate-ammonium at a similar level (see Ellgehausen, 1986a) (Ellgehausen, 1986b).

The proposed metabolic pathway of glufosinate-ammonium is depicted in Figure 2.

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

The results of studies of acute toxicity with glufosinate-ammonium are summarized in Table 8.

Figure 2. Proposed metabolic pathway of glufosinate-ammonium in the rat

glufosinate ammonium (GA)

Oxidative Deamination

Acetylation (reversible)

4-methylphosphinico-2-oxo-butanoic acid (PPO) [transient]

N-acetyl glufosinate (NAG) [minor]

Decarboxylation

3-methylphosphinico-propionic acid (MPP) [major]

2-hydroxy-4-methylphosphinico-butanoic acid (MHB) [minor]

Oxidation, Decarboxylation

2-methylphosphinico-acetic acid (MPA) [very minor]

4-methylphosphinico-butanoic acid (MPB) [minor]

Table 8. Results of studies of acute toxicity with glufosinate-ammonium

Species	Strain	Sex	Route	Vehicle	Purity (%)	LD_{50} (mg/kg bw) / LC_{50} (mg/l)	Reference
Mouse	NMRI	M	Oral	Deionized water	92.1	431	Mayer & Weigand (1980a)[a]
Mouse	NMRI	F	Oral	Deionized water	92.1	416	Mayer & Weigand (1980b)[a]
Mouse	ICR	M/F	Oral	Saline	92.1	436 (M) 464 (F)	Inoue (1982)[b]
Rat	Wistar	M	Oral	Deionized water	92.1	2000	Mayer & Weigand (1980c)[c]

Species	Strain	Sex	Route	Vehicle	Purity (%)	LD_{50} (mg/kg bw) / LC_{50} (mg/l)	Reference
Rat	Wistar	F	Oral	Deionized water	92.1	1620	Mayer & Weigand (1980d)[c]
Rat	Fischer	M	Oral	Distilled water	92.1	1660	Ohtaka, Takahashi & Nakayoshi (1981a)[d]
Rat	Fischer	F	Oral	Distilled water	92.1	1510	Ohtaka, Takahashi & Nakayoshi (1981b)[d]
Rat	Wistar	M/F	Oral	Distilled water	50[e]	> 2000 (M) > 2000 (F)	Tavaszi (2011a)[f]
Dog	Beagle	M/F	Oral	Deionized water	92.1	200–400	Mayer & Kramer (1980)[g]
Rat	Wistar	M	Dermal	Deionized water	97.2	> 4000	Mayer & Weigand (1982a)[h]
Rat	Wistar	F	Dermal	Deionized water	97.2	> 4000	Mayer & Weigand (1982b)[h]
Rat	Wistar	M/F	Dermal	—	50[e]	> 2000	Zelenák (2011a)[i]
Rabbit	New Zealand White	M/F	Dermal	Distilled water	96.9	> 2000 (M) 1500–2000 (F)	Kynoch & Parcell (1986)[j]
Rat	Wistar	M/F	Inhalation	—	95.3	1.26 mg/l dust 2.60 mg/l dust	Hollander & Weigand (1985)[k]
Rat	Wistar	M/F	Inhalation	—	50[e]	> 5.0 mg/l	Grosz (2011)[l]

F, female; LC_{50}, median lethal concentration; LD_{50}, median lethal dose; M, male

[a] Performed according to Organisation for Economic Co-operation and Development (OECD) Test Guideline No. 401. Doses of 315, 500 and 800 mg/kg bw were used. Dose-dependent mortality was observed at all doses. The following clinical signs were observed: ataxia, bizarre movements, squatting, abdominal position, clonic convulsions, convulsive jumping and rolling spasms, "Straub" phenomenon and irregular, jerky respiration.

[b] Doses of 231–857 mg/kg bw were used. At all doses, males and females exhibited clinical signs. Observed clinical signs were decreased spontaneous motility, clonic convulsions, a ventral or abdominal posture, ataxic gait, piloerection and loss of coat lustre. Dose-dependent mortality was observed at doses of 300 mg/kg bw and higher.

[c] Performed according to OECD Test Guideline No. 401. Doses of 630–3150 mg/kg bw were used. Males at doses of 1000 mg/kg bw and higher and females at doses of 1600 mg/kg bw and higher exhibited hyperreflexia, Dalrymple's sign, exophthalmus, squatting, straddled legs and retracted abdomen or flank, hyporeflexia, bristled hair, poor general condition, ataxia, lateral position, blood crusted eyes and snouts, decreased and irregular respiratory rate, passiveness, disequilibrium, high-legged posture, abdominal and lateral position, trembling, convulsions, clonic convulsions and rolling spasms. Dose-dependent mortality was observed from 1000 mg/kg bw in males and from 1600 mg/kg bw in females. No symptoms of intoxication were seen in males or females from the 630 mg/kg bw dose group or females dosed at 1000 mg/kg bw.

[d] Performed according to OECD Test Guideline No. 401. Doses of 1000–3000 mg/kg bw were used. Every dose group exhibited occurrences of quietness, hypersensitivity to touch, salivation, lacrimation, ventral position and piloerection. At doses of 1600 mg/kg bw and higher in males and 2190 mg/kg bw and higher in females, diarrhoea and hypersensitivity to touch manifested as jumping and convulsions were seen. Dose-dependent mortality was observed from 1170 mg/kg bw in both sexes.

[e] Fifty per cent technical concentrate.

[f] Performed according to OECD Test Guideline No. 423. Doses of 300 and 2000 mg/kg bw were used. No clinical signs or mortality were observed.

Table 8 (continued)

[g] Performed according to OECD Test Guideline No. 401. Doses of 200 and 400 mg/kg bw were used. At 200 mg/kg bw, dogs showed squatting, benumbedness, trembling, diarrhoea and disequilibrium, abdominal position, retracted flank, hyporeflexia, increased lacrimation, salivation and rhinorrhoea, paraesis or paralysis of the hindlegs and discoloration of the tongue (bluish) and ocular and oral mucosae (distinctly reddened). During irregular intervals, both animals showed quick turning of the body on the hindlegs (rotary or arena motions). In addition, periodic extension spasms and marked dyspnoea accompanied by sounds in the form of loud cries were observed. During the following 8 days, all symptoms of intoxication were regressive and completely reversible by the termination of the experiment. At 400 mg/kg bw, both dogs died 2–3 days after treatment. The symptoms of intoxication in the male dog largely corresponded to those observed in the 200 mg/kg bw group, with additional symptoms of ataxia, emesis and noisy, jerky respiration. The female animal proved to be distinctly more sensitive and showed ataxia, abdominal position, tonoclonic convulsions, orthotonus, opisthotonus, miosis and noisy, jerky respiration. The body temperature of both animals was within the normal range 24 hours after dosing.

[h] Performed according to OECD Test Guideline No. 402. No symptoms of intoxication were observed in male rats in the 2000 mg/kg bw group. Males dosed at 4000 mg/kg bw showed hyperactivity, convulsions, retracted abdomen and sides, Dalrymple's sign, increased salivation and aggressivity. In females, the following symptoms were observed: hyperactivity, passiveness, lethargy, loss of equilibrium, squatting, abnormal posture, abdominal position, trembling, convulsions, retracted abdomen and sides, convulsive jumping, "Straub" phenomenon, bristled hair, Dalrymple's sign or blepharophimosis, increased salivation, blood-coloured urine, aggressivity, masticator movements, emaciation and poor general condition.

[i] Performed according to OECD Test Guideline No. 402 with glufosinate-ammonium technical, a liquid. No clinical signs or dermal signs were observed.

[j] Performed according to United States Environmental Protection Agency (USEPA) Guideline 81-2 (resembles OECD Test Guideline No. 402). Clinical signs of intoxication were piloerection, unsteady gait, lethargy, ataxia and prostration. There were no dermal reactions at the site of application in any of the treated animals.

[k] Performed according to USEPA Guideline 81-3 (resembles OECD Test Guideline No. 403). Clinical signs of intoxication were observed at all dose levels and included narrowed eyes, periodic tremors and clonic convulsions, hyperactivity, piloerection, increased salivation and passivity.

[l] Performed according to OECD Test Guideline No. 403 At 5.0 mg/l, slightly to moderately laboured respiration and slightly increased respiratory rate were the main clinical signs observed following the exposure on day 0. On the following days (days 1–4), toxicologically relevant clinical signs were slightly laboured respiration (all animals), weak body condition (all animals), slightly to severely decreased activity (3/5 males and 3/5 females), increased irritability (5/5 males and 3/5 females) and slight to moderate ataxia (1/5 males and 3/5 females). These signs were no longer observed after day 4, except for a single male and female. In the male rat, the clinical signs ceased on day 5, whereas weak body condition was observed up to day 13. In the female rat, the majority of clinical signs persisted up to death (day 8).

(b) Dermal irritation

In an acute dermal irritation study using six New Zealand White rabbits (sex not specified), the intact skin as well as the abraded skin were exposed for 24 hours under semi-occlusion to 0.5 g glufosinate-ammonium (purity not reported) moistened with 0.1 ml of 0.9% saline. Dermal irritation was scored according to the Draize system at 24, 48 and 72 hours after patch removal.

One animal exhibited erythema of the intact skin and four animals exhibited erythema of the abraded skin immediately after patch removal. No oedema was observed in any animal at any time point. Glufosinate-ammonium was non-irritating to rabbit skin (Mayer & Weigand, 1982c).

In an acute dermal irritation study, performed in accordance with Organisation for Economic Co-operation and Development (OECD) Test Guideline No. 404, the intact skin of three male New Zealand White rabbits was exposed for 4 hours under semi-occlusion to 0.5 ml of glufosinate-ammonium 50% technical concentrate. Dermal irritation was scored according to the Draize system at 1, 24, 48 and 72 hours after patch removal. Statements of adherence to QA and GLP were included.

Very slight oedema was observed in two animals at 1 and 24 hours after patch removal. Erythema and oedema were also recorded in one animal at 48 hours after patch removal. Glufosinate-ammonium 50% technical concentrate was non-irritating to rabbit skin (Zelenák, 2011b).

(c) Ocular irritation

In an acute eye irritation study, performed according to OECD Test Guideline No. 405, 0.0690 g (0.1 ml weight equivalent) of glufosinate-ammonium (purity 95.2%) was instilled into the conjunctival sac of the right eye of four male and two female New Zealand White rabbits. The untreated left eye served as a control. The eyes were macroscopically examined for signs of irritation according to the Draize system at 1, 24, 48 and 72 hours (all animals) and at day 7 (five animals) and day 10 (two animals) post-instillation. Statements of adherence to QA and GLP were included.

The test article produced iritis in five of six test eyes at the 1-hour scoring interval. The iridial irritation was completely reversed by the 24-hour scoring interval. Conjunctivitis (redness, swelling and discharge) was noted in all (6/6) test eyes at the 1-hour scoring interval. Conjunctival irritation diminished over the remainder of the study and was no longer evident in any animals by study day 10. Under the conditions of this study, glufosinate-ammonium was not irritating to the eyes of rabbits (Merriman, 1995).

In an acute eye irritation study, 0.1 g of glufosinate-ammonium (purity not reported) premixed with one drop of 0.9% sodium chloride solution was placed into the conjunctival sac of the left eye of nine New Zealand rabbits (sex not specified). One minute after the application of the test substance, the eyes of three animals were flushed with physiological saline. The eyes of the remaining six animals were flushed after 24 hours. The eyes were macroscopically examined for signs of irritation at 1, 24, 48 and 72 hours after application, using a scale similar to the system of Draize.

In the rabbits whose eyes were rinsed 24 hours after application, slight signs of irritation of the iris (at 1 hour), redness of the conjunctivae (at 1–48 hours), swelling of lids and nictitating membranes (at 1–7 hours) and discharge with moistening of the lids (at 1–7 hours) were observed. All animals had recovered by day 3. Irritation of the iris and conjunctivae was also observed when the eye was rinsed 1 minute after application of the substance, although to a lesser degree. Under the conditions of this study, glufosinate-ammonium was not irritating to the eyes of rabbits (Mayer & Weigand, 1982c).

In an acute eye irritation study, 0.1 ml of glufosinate 50% technical material was placed into the conjunctival sac of the left eye of three male New Zealand rabbits. The eyes were macroscopically examined for signs of irritation according to the Draize system at 1, 24, 48 and 72 hours after application. Statements of adherence to QA and GLP were included.

One hour after the application, conjunctival redness (score 1, two animals), conjunctival discharge (score 2, two animals) and conjunctival chemosis (score 1, 1 animal) were observed. At 24 hours after treatment, conjunctival redness (score 1, two animals) and conjunctival discharge (score 1, one animal) were observed. At 48 hours after treatment, one animal displayed conjunctival redness. At 72 hours after treatment, no clinical signs were observed. Under the conditions of this study, glufosinate-ammonium was not irritating to the eyes of rabbits (Tavaszi, 2011b).

(d) Dermal sensitization

A dermal sensitization study (local lymph node assay) was performed according to OECD Test Guideline No. 429. CBA/J female mice (4–5 per group) were treated for 3 consecutive days with glufosinate-ammonium 50% technical concentrate at a concentration of 10%, 25% or 50% in vehicle (1% Pluronic Acid L92® in water). The test substance was applied in a volume of 25 µl to the external surface of each ear. A positive control group (four animals) received 30% α-hexylcinnamaldehyde in vehicle. The draining auricular lymph nodes were examined for T-lymphocyte proliferation, as measured by incorporation of tritiated thymidine. Statements of adherence to QA and GLP were included.

Four out of five animals treated with glufosinate-ammonium at 50% and one out of five animals treated with glufosinate-ammonium at 25% were found dead on day 3. No clinical signs were observed in these animals. No mortality and no clinical signs were observed in the other study groups. The isotope incorporation was less than 3-fold at treatment concentrations of 10% and 25% compared with the vehicle control. Treatment with the positive control, α-hexylcinnamaldehyde, resulted in a 19-fold isotope incorporation. Under the conditions of this study, glufosinate-ammonium was not sensitizing (Repetto, 2011).

A dermal sensitization study (Buehler test) with glufosinate-ammonium (purity 95.3%) was performed according to United States Environmental Protection Agency (USEPA) Guideline 81-6. In a preliminary study, 0.5 ml of test substance dissolved in 0.9% saline at a concentration of 50%, 5% or 0.5% was applied to the shaven flanks of female Pirbright-White guinea-pigs (two animals per dose). After 24 hours, no skin irritancy was observed; therefore, the highest concentration was chosen for the definitive study.

In the main study, the shaven left flanks of 20 female guinea-pigs were induced by 6-hour occluded topical applications of 50% glufosinate-ammonium in saline on days 1, 3, 5, 8, 10, 12, 15, 17 and 19. A control group of 10 animals remained untreated during the induction phase. On day 37, all test and control animals were challenged by 6-hour occluded topical applications of 50% glufosinate-ammonium to the shaven right flank. Dermal responses were assessed approximately 24 and 48 hours after application. A positive control was not included. Statements of adherence to QA and GLP were included.

No irritant effects on the treated sites were noted during the induction phase. Challenge treatment on day 37 produced no irritant effects on the treated skin sites at 24 and 48 hours after treatment. Under the conditions of this study, glufosinate-ammonium was not a skin sensitizer (Rupprich & Weigand, 1983).

In a dermal sensitization study using the Magnusson and Kligman maximization test, performed in accordance with OECD Test Guideline No. 406, glufosinate-ammonium (purity 95.2%) was tested in 20 female Pirbright-White guinea-pigs. A preliminary study established 1% and 50% test substance concentrations in saline as suitable for the intradermal induction and dermal induction and challenge phases, respectively. The control group consisted of 10 animals. In the first induction phase, the animals were subjected to two intradermal injections of 50% Freund's Complete Adjuvant, 1% glufosinate-ammonium in isotonic saline and 1% glufosinate-ammonium in 50% Freund's Complete Adjuvant. Seven days later, the same area of skin was treated by topical application of 0.5 ml of a 50% solution of glufosinate-ammonium in saline and the test site covered with an occlusive dressing for 48 hours. The same induction procedure was carried out on control groups with vehicles only. On day 22, all animals were challenged by a 24-hour occluded topical application of 50% glufosinate-ammonium in saline. The test sites were assessed 24 and 48 hours after removal of the occlusive bandages. Benzocaine was used as a positive control. Statements of adherence to QA and GLP were included.

The intradermal injections with Freund's Complete Adjuvant (with and without test substance) caused severe erythema and oedema as well as indurations and encrustations. The application sites treated with the test substance showed slight erythema. Because of these strong irritation reactions of the skin, a local irritant such as sodium dodecylsulfate was not applied at day 7.

Following dermal induction, removal of the patches at day 10 revealed severe erythema and oedema with indurated and encrusted skin at the sites previously treated with Freund's Complete Adjuvant. The application sites treated with the test substance showed slight erythema and oedema. The vehicle alone showed no signs of irritation.

After the dermal challenge treatment on day 22, no signs of irritation were observed in the control and the treated groups 24 and 48 hours after the removal of the occlusive bandage. None of

the 20 animals in the treated group showed a positive skin response after the challenge procedure. Under the conditions of this study, glufosinate-ammonium was not a skin sensitizer (Hammerl, 1996).

2.2 *Short-term studies of toxicity*

(a) Oral administration

Mice

In a 90-day dietary range-finding study, groups of 10 male and 10 female NMRI mice were administered glufosinate-ammonium (purity 95.5%) at 0, 1750, 3500 or 7000 ppm. The low and middle doses were equal to 274 and 561 mg/kg bw per day for males and 356 and 644 mg/kg bw per day for females, respectively. The mice of the high-dose group died within 8 days. Animals were observed daily for mortality and clinical signs. Feed consumption and body weights were recorded weekly. At the end of treatment, blood samples were collected (five animals of each sex per group for haematology and five other animals of each sex per group for clinical biochemistry). All surviving animals were necropsied, adrenals, kidneys, liver and testes were weighed and a wide range of organs and tissues was histologically examined. Statements of adherence to QA and GLP were included.

At 7000 ppm, all the animals died between days 4 and 8 of treatment. At 3500 ppm, four males and five females died between days 6 and 11 of treatment, whereas another male died at day 45. At 1750 ppm, one female died on day 8 of treatment. The death of a 1750 ppm male occurred after blood sampling and was not considered to be treatment related. At 3500 and 7000 ppm, ruffled fur, sedation, ventral recumbence or hunched posture, and emaciation were mainly noted. At 1750 ppm, ruffled fur was evident in all animals, and sedation and emaciation were seen in some females. Apart from ruffled fur, the clinical signs were observed only during the first part of the study. A body weight reduction was observed in mice of the low-dose (4–8%) and mid-dose groups (14–20%) during the 1st week of treatment. Statistically significant reduced body weights were observed in the low-dose males during the first 4 weeks of treatment, mid-dose males throughout the treatment period and mid-dose females during the first 2 weeks of treatment. Feed consumption showed a pattern similar to body weight gain. At 1750 and 3500 ppm, no toxicologically relevant effects on haematology or clinical chemistry were found. Necropsy revealed no toxicologically relevant gross findings, effects on organ weights or histopathological changes (Dotti, Luetkemeier & Biedermann, 1994).

Technical glufosinate-ammonium was administered via the diet to groups of 10 male and 10 female NMRI mice for 13 weeks at a dietary level of 0, 80, 320 or 1280 ppm (equal to 0, 17, 67 and 278 mg/kg bw per day for males and 0, 19, 87 and 288 mg/kg bw per day for females, respectively). Animals were examined daily for mortality and clinical signs. A detailed clinical examination was performed weekly. Feed consumption and body weights were recorded weekly. Ophthalmological examinations and hearing tests were performed prior to and at the end of treatment. Haematology and clinical chemistry were performed on five animals of each sex per dose at the end of the treatment period. A complete necropsy was performed on all mice. Tissues and organs were examined macroscopically. Selected organs were weighed and examined microscopically. Statements of adherence to QA and GLP were included.

No treatment-related effects on mortality, clinical signs, body weight changes, feed consumption or ophthalmology were noted. At the high dose, slightly higher aspartate aminotransferase (AST) activities (34%) in males and alkaline phosphatase (ALP) activities (38%) in females were noted. As no histopathological changes were observed in the liver, these increases are considered not adverse. A slight, but statistically significant, dose-related increased potassium concentration was noted in the males at 320 ppm (19%) and 1280 ppm (24%). Haematological investigations showed statistically significant reductions of total leukocyte count (white blood cells) for males at 320 ppm (44% decrease) and 1280 ppm (41% decrease) and of erythrocyte count (red blood cells) (5–6% decrease) and haematocrit (4% decrease) in females at 320 and 1280 ppm. A

statistically significant reduction (33–44%) of segmented neutrophils was noted for all treated females. As no corresponding histological changes were observed and as no haematological effects were observed in a study using higher doses or a study of longer duration, these findings are considered not toxicologically relevant. Although statistically significant, a slightly increased relative liver weight (8%) in high-dose males was not considered adverse. No treatment-related macroscopic or histopathological changes were found.

The NOAEL was 1280 ppm (equal to 278 mg/kg bw per day), the highest dose tested (Suter, 1984a).

Rats

In a dietary range-finding study, Wistar rats (five of each sex per group) were fed diets containing glufosinate-ammonium (purity 95.3%) for 4 weeks at a concentration of 0, 50, 500, 2500 or 5000 ppm (equal to 0, 5, 53, 276 and 534 mg/kg bw per day for males and 0, 6, 58, 271 and 557 mg/kg bw per day for females, respectively). Rats were examined daily for clinical signs of toxicity. Body weight and feed consumption were measured weekly during the study. Haematology, serum biochemical determinations and urine analyses were performed after 4 weeks. At the end of the treatment period, the rats were necropsied, liver and kidneys were weighed and kidneys were examined microscopically. Acetylcholinesterase activity was assessed in brain and erythrocytes. Statements of adherence to QA and GLP were included.

No deaths or clinical signs of systemic toxicity were observed. No toxicologically relevant effects were observed on body weight gain, feed consumption, haematology, clinical chemistry, urine analysis or pathology (Suter, 1984b).

In a 90-day dietary study, groups of 30 male and 30 female Fischer rats received glufosinate-ammonium (purity 92.1%) at a concentration of 0, 8, 64, 500 or 4000 ppm (equal to 0, 0.52, 4.1, 32 and 263 mg/kg bw per day for males and 0, 0.63, 4.8, 39 and 311 mg/kg bw per day for females, respectively). Twenty animals of each sex per group were sacrificed at the end of the 13-week study period, and 10 animals of each sex per group were maintained for a 4-week post-dosing recovery period. Animals were checked daily for clinical signs of toxicity. Body weights, feed consumption and water consumption were measured weekly. Ophthalmological examinations were carried out on high-dose rats before dosing, on day 47 and on the last day of dosing. Blood samples were taken from 10 animals of each sex per group and sampled for haematology and clinical biochemistry on days 47 and on the last day of dosing. Urine analysis was performed on samples taken from 10 animals of each sex per group on days 47 and 86 of dosing and on day 23 of the recovery period. At necropsy, selected organs were weighed and examined macroscopically and histopathologically.

No treatment-related mortality or clinical signs were seen. Reduced body weight gain (up to 11%) and feed consumption (up to 26%) were observed in both sexes in the 4000 ppm group for the first 2–3 weeks of treatment, in particular during the 1st week of treatment. The study authors attributed this to poor palatability of the test substance. Final body weights were not affected. No toxicologically relevant changes in ophthalmoscopy, haematology, clinical chemistry or histopathology were seen. Urine analysis demonstrated a lower pH in urine of high-dose rats. In treated males, statistically significant increases in absolute (all doses, 7–19%) and relative (at doses ≥ 64 ppm, 3–15%) kidney weights were observed. In females, statistically significant increases in absolute (11%) and relative (7%) kidney weights were observed at the high dose only. Such increases were still observed at the end of the recovery period, although to a lesser extent. As the increases in kidney weights were not accompanied by histopathological changes in the kidneys and were not observed in other studies, they are considered not related to treatment.

The NOAEL was 4000 ppm (equal to 263 mg/kg bw per day), the highest dose tested (Ohtaka, Takahashi & Nakayoshi, 1982).

In a 13-week dietary toxicity study, glufosinate-ammonium (purity 95.5%) was administered to groups of 10 male and 10 female SPF bred Hanover-derived Wistar rats at 0, 7500, 10 000 or 20 000 ppm (equal to 0, 521, 686 and 1351 mg/kg bw per day for males and 0, 574, 741 and 1443 mg/kg bw per day for females, respectively). Animals were checked daily for clinical signs of toxicity. Body weights, feed consumption and water consumption were measured weekly. The rats were tested in a functional observational battery before treatment and at weeks 1, 2, 3, 4, 8 and 13. Ophthalmological examinations were carried out before dosing and at week 13. Blood was sampled for haematology and clinical biochemistry at the end of the treatment period. All animals were necropsied, and all gross lesions and a wide range of tissues of rats of the control and high-dose groups and the brain, spinal cord, and left and right sciatic and tibia nerves from rats of the low- and mid-dose groups were examined microscopically. Statements of adherence to QA and GLP were included.

At 20 000 ppm, one female died on each of day 6 and day 8. These deaths were considered to be treatment related. In the first weeks of treatment in the high-dose group, sedation, lateral recumbency, hunched posture, dyspnoea, ruffled fur and emaciation were noted in both sexes. In addition, in a few females, spasms and lacrimation were noted. Ophthalmoscopic examinations revealed no treatment-related effects. For the first 2 weeks of treatment, reduced body weight gain was observed in males of the low-dose group (up to 15%), mid-dose group (up to 13%) and high-dose group (up to 24%) and in females of the high-dose group (up to 19%). In fact, high-dose males and females showed a 6–7 g body weight loss during the 1st week of treatment, while control rats gained 19–44 g over this period. Final body weights were not affected. Mean feed consumption over the entire test period was reduced in the males by about 10% in the low- and mid-dose groups and by about 15% at 20 000 ppm and in high-dose females by about 10%. The effects on feed consumption were mainly evident during the first half of treatment and in particular during weeks 1 and 2 of treatment.

Functional observational battery testing showed miosis and a slight decrease in exploratory activity, alertness and/or startle response, particularly in the early stages of treatment, in the low-dose rats. In addition, mid-dose rats showed increased body tone, increased pain response and fearfulness and occasionally rearing with convulsive twitches and perfuse salivation. In high-dose rats, signs of abnormal behaviour were similar to those seen in animals at 10 000 ppm, although more severe and persistent. In addition, diarrhoea, increased vocalization and apathy were recorded.

Haematology showed small, but statistically significant, reductions in erythrocyte count in low-dose males and mid- and high-dose males and females. Mean corpuscular volume was slightly increased (4%) in males at 10 000 and 20 000 ppm. Increases in high (140–160%) and middle fluorescent reticulocyte ratios (22–25%) were observed in mid- and high-dose males. Low fluorescent reticulocyte ratios were decreased (11–16%) in males of all treatment groups. Changes in haematocrit and reticulocyte count were not dose dependent.

Necropsy and histopathological examination did not reveal treatment-related findings.

The lowest-observed-adverse-effect level (LOAEL) was 7500 ppm (equal to 521 mg/kg bw per day), based on reduced body weight gain and feed consumption, reductions in erythrocyte count and low fluorescent reticulocyte ratios in males and miosis and a slight decrease in exploratory activity, alertness and/or startle response observed in the functional observational battery in both sexes (Dotti, Luetkemeier & Powell, 1993).

Dogs

In a 28-day oral range-finding study in Beagle dogs, the mode of action and toxicokinetics of glufosinate-ammonium were explored. Groups of six male and six female dogs received glufosinate-ammonium (purity 95.3%) in gelatine capsules at a dose of 0, 1 or 8 mg/kg bw per day for 28 days, followed by a 4-day recovery period. From days 19 to 28, the dogs received ^{14}C-labelled glufosinate-ammonium (radiochemical purity 98%) in distilled water. One dog of each sex per dose group was killed on day 18, 19 or 28 of the treatment period or on day 1, 2 or 4 post-treatment. Dogs were

observed daily for mortality and clinical signs. Feed consumption was measured daily and body weight weekly during the study. The dogs in the control and high-dose groups were subjected to neurobehavioural assessments before dosing and on days 1, 2, 3, 4, 8, 11, 15, 18, 22 and 25. Animals of the low-dose group were subjected to neurobehavioural assessments before dosing and on days 4, 11, 18 and 25. The dogs were subjected to ophthalmoscopy (pretest and on days 14 and 25), haematology, blood chemistry (including catecholamine measurements) and urine analysis (pretest, on days 11 and 28 of treatment and on day 4 post-treatment). At necropsy, liver, heart, kidney, spinal cord and four brain regions (cortex, midbrain, cerebellum and brain stem) were collected, weighed and examined. Glutamine synthetase activity and free amino acid levels in different tissues were measured. Statements of adherence to QA and GLP were included.

No animals died during the study. A slight increase in spontaneous motor activity in high-dose animals was observed. Detailed observations and neurological examinations showed no treatment-related effects. Ophthalmological examinations showed no treatment-related findings. The body weight gain and feed consumption of the high-dose males were reduced during the 1st week of treatment, and those of the corresponding females were reduced during the entire treatment phase. No treatment-related effects on haematology, clinical biochemistry, urine analysis, organ weights or macroscopy were observed. In high-dose animals, inhibition of glutamine synthetase activity in midbrain, cerebellum and spinal cord (males only) was observed. Reductions in glutamine synthetase activity in spinal cord of females and in cortex and liver of both sexes were not statistically significant (Table 9). Glutamine synthetase activity in kidney was not affected.

Table 9. Mean levels of glutamine synthetase activity in tissues

Sex	Tissue	0 mg/kg bw per day	1 mg/kg bw per day		8 mg/kg bw per day	
		Mean[a]	Mean	% of control	Mean	% of control
Males	Liver	6.41	6.06	95	3.98	62
	Midbrain	4.52	4.49	99	2.93*	65
	Cerebellum	3.64	3.58	98	1.72*	47
	Cortex	6.92	7.17	104	5.09	74
	Spinal cord	1.10	0.90	82	0.55*	50
Females	Liver	5.96	6.24	105	4.19	70
	Midbrain	4.44	4.88	110	3.20*	72
	Cerebellum	3.22	3.39	105	2.16*	67
	Cortex	6.37	6.78	106	5.87	92
	Spinal cord	1.01	1.05	104	0.70	69

From Sachsse (1986a)

* $P < 0.05$ (Dunnett test based on pooled variance or Steel test)

[a] One dog of each sex per dose group was killed on day 18, 19 or 28 of the treatment period or on day 1, 2 or 4 post-treatment. The data are the means of these six dogs of each sex per dose. Glutamine synthetase activity is expressed as micromoles of γ-glutamyl-hydroxamate formed per millilitre reaction mixture per 20 minutes.

In cerebellum of high-dose animals, an increased level of α-ketoglutarate was found. In cerebellum, the levels of taurine and phosphoethanolamine were significantly reduced at the highest dose in both sexes. Glutamate, aspartate, cysteine, γ-aminobutyric acid, glycine and β-alanine levels in neural tissue and brain cholinesterase activity were not affected by treatment.

The NOAEL was 1 mg/kg bw per day, based on the reductions in glutamine synthetase activity in the central nervous system, a slight increase in spontaneous motor activity and a reduction in body weight gain and feed consumption at 8 mg/kg bw per day (Sachsse, 1986a).

In a 90-day toxicity study, four male and four female Beagle dogs per dose group received feed containing glufosinate-ammonium (purity 92.1%) at a dietary level of 0, 4, 8, 16, 64 or 256 ppm (equal to 0, 0.13, 0.26, 0.53, 2.0 and 7.8 mg/kg bw per day, respectively). Animals were checked daily for viability and clinical signs. Feed and water consumption and body weights were measured weekly. Ophthalmological examinations were performed in week 12. Blood samples for haematology and clinical chemistry were collected pretest and in weeks 7 and 13. A liver function test (bromosulfophthalein [BSP] method) was carried out on all dogs of the two highest-dose groups and the control group during week 13; a kidney function test (phenolsulfonphthalein [PSP] method) was conducted on the two highest-dose groups and the control group during week 12. Urine analysis was performed pretest and in weeks 7 and 12. Control and 256 ppm dogs were subjected to electrocardiography (ECG) pretest and at week 13. All dogs were necropsied, and weights of heart, spleen, brain, liver, pituitary, adrenals, kidneys, testes, ovaries, thymus, thyroid with parathyroid and lungs were recorded. Histology was performed on a large selection of organs from all dogs. Statements of adherence to QA and GLP were included.

No mortality or clinical signs of toxicity were observed. Feed consumption was 14% lower in high-dose (256 ppm) females. Water intake was similar in all groups. In high-dose (256 ppm) females, a statistically significant reduction in body weight gain (10%) was observed during the second half of the study. Ophthalmological and ECG examinations revealed no treatment-related effects.

No consistent effects on haematological or urine analysis parameters were observed. Slight reductions in plasma inorganic phosphate levels in some treatment groups at week 7 were not accompanied by changes in calcium concentrations or abnormalities in the histopathology of the parathyroid or kidneys and were attributed to the relatively high control values at week 7.

At week 13, direct plasma bilirubin levels were relatively low in males of all dose groups but showed no dose–response relationship. This finding was not accompanied by any changes in total bilirubin levels and was considered not to be of toxicological significance. Plasma PSP concentrations were relatively low in males as well as in females of the two highest-dose groups, but within the historical control range. As an impaired renal function is reflected by an increase rather than a decrease in PSP concentration and as no treatment-related pathological changes were observed in the kidneys, the reduction in PSP levels is considered not toxicologically relevant. Other isolated changes in clinical chemistry parameters were considered not to be of toxicological significance.

Microscopic and histopathological examination and organ weight measurements did not reveal effects related to glufosinate-ammonium treatment.

The NOAEL was 64 ppm (equal to 2.0 mg/kg bw per day), based on the reduction in body weight gain and feed consumption in females at 256 ppm (equal to 7.8 mg/kg bw per day) (Lina et al., 1982).

In a 1-year study, glufosinate-ammonium was administered to groups of eight male and eight female Beagle dogs via the diet at 0, 60, 150 or 250 ppm. The achieved intake levels were 0, 1.8, 4.5 and 8.4 mg/kg bw per day, respectively, except for the first 10–17 days of the study, when high-dose animals received 375 ppm, equal to 10.6–13.6 mg/kg bw per day for males and 15.4–16.0 mg/kg bw per day for females. As this was above the target dose of 8.5 mg/kg bw per day, the dietary concentration was lowered to 250 ppm from day 12 onward. Four dogs of each sex per dose group were assigned for interim kill after 6 months of treatment, and four animals of each sex per group were assigned for terminal kill after 1 year of treatment. Animals were examined for mortality and clinical signs. Feed consumption was recorded daily, and body weights were recorded weekly. Ophthalmoscopy and hearing tests and examinations of mucous membranes and teeth were performed pretest and after 3, 6, 9 and 12 months. ECG was performed pretest and at 6 or 12 months. Haematology, clinical chemistry and urine analysis were performed pretest and after 1, 3, 6 and 12 months. The BSP clearance test for measuring liver function and the PSP clearance test for measuring

kidney function were performed after 6 and 12 months of treatment. The dogs were necropsied, and weights of heart, spleen, brain, liver, pituitary, adrenals, kidneys, testes, ovaries, thymus, thyroid with parathyroid and lungs were recorded. Histology was performed on a large selection of organs and tissues from all dogs. Bone marrow was examined histologically in control and high-dose animals only. Statements of adherence to QA and GLP were included.

One male and one female from the high-dose group were found dead on days 14 and 10, respectively. In these two animals and an additional high-dose female, trismus, salivation and hyperactivity followed by somnolence and hypoactivity were observed immediately after feed consumption, as well as stereotypic stiff gait, tremor, ataxia, whining, urinating, tonoclonic spasm, paddling movements, opisthotonus and lateral recumbence. These clinical signs were first seen after 9 days of treatment. The deaths of the two high-dose dogs were caused by heart and circulatory failure attributed to marked myocardial necrosis in one dog and to slight myocardial necrosis and severe necrotizing aspiration pneumonia in the other dog and were considered treatment related. In the other animals, no treatment-related effects on feed consumption, body weight gain, hearing, teeth, mucous membranes, ophthalmoscopic examination, ECG, haematology, clinical chemistry, macroscopy, histopathology including bone marrow and organ weights were observed.

The NOAEL was 150 ppm (equal to 4.5 mg/kg bw per day), based on clinical signs of toxicity and mortality observed at 375 ppm (equal to 10.6–13.6 mg/kg bw per day) during the first 2 weeks of the study. The study indicates that glufosinate-ammonium has a steep dose–response curve in dogs (Bathe, 1984).

(b) Dermal application

Groups of six male and six female Wistar rats were dermally exposed 6 hours/day, 5 days/week, to a 0%, 7.5%, 22.5% or 75% solution of technical glufosinate-ammonium (purity 95.3%) in deionized water, equal to doses of 0, 100, 300 and 1000 mg/kg bw per day. Twenty-one applications were given over a 30-day period. Additionally, two groups of five males and five females, one control and one dosed at 1000 mg/kg bw per day, were treated by a similar regimen followed by a 14-day recovery period. The behaviour and the general health and condition of the animals were observed daily. Body weights and feed consumption were recorded twice weekly, and water consumption was recorded once weekly. Haematology, clinical chemistry and urine analysis were performed at the end of the study. At termination of the study, all animals were killed and necropsied. Weights of heart, spleen, brain, liver, pituitary, adrenals, kidneys, testes, ovaries, thyroid and lungs were recorded. Histology was performed on a selection of organs and tissues. Statements of adherence to QA and GLP were included.

No dermal irritation was noted. At 300 mg/kg bw per day, one male showed aggressive behaviour, squatting position, piloerection and convulsive jumping and rolling spasms at the end of the treatment period. At the high dose, four male and two female animals showed either timid or aggressive behaviour, increased motor excitation (especially following tactile stimuli), piloerection or squatting position. One male refused its feed almost entirely from the beginning of the 2nd week of treatment and was removed in an emaciated condition on day 16. Body weight gain and feed and water consumption were not affected by treatment.

Occasional changes in haematology and clinical chemistry in the mid- and high-dose groups were not dose related and/or were within the range of normal variation and were considered not to be treatment related. Urine analysis was normal for all groups. Therefore, examination of haematological, clinical chemistry and urinary parameters was not performed in the recovery animals. Macroscopic, histopathological and organ weight examinations showed no toxicologically significant effects of glufosinate-ammonium treatment.

The NOAEL was 100 mg/kg bw per day, based on clinical signs observed in a male at 300 mg/kg bw per day (Ebert & Kramer, 1985a).

(c) Exposure by inhalation

Groups of 15 male and 15 female Wistar rats were exposed nose-only to dust particulate aerosol atmospheres of glufosinate-ammonium (purity 95.3%) at a concentration of 0, 0.012, 0.025 or 0.050 mg/l air, 6 hours/day, 5 days/week, over a 40-day period. The control animals received air only. About 82–85% of the total aerosol had a mass median aerodynamic diameter (MMAD) of less than 7 µm, and 46–49% had an MMAD of less than 3 µm. The behaviour and the general health and condition of the animals were observed daily. Body weights and feed consumption were recorded twice weekly, and water consumption was recorded once weekly. Haematology and clinical chemistry were performed at the end of the study. One day after the last exposure, 10 males and 10 females from each group were killed and necropsied. The remaining rats were killed and necropsied after a 29-day recovery period. Weights of heart, spleen, brain, liver, pituitary, adrenals, kidneys, testes, seminal vesicles, ovaries, thyroid and lungs were recorded. Histology was performed on a large selection of organs and tissues. Statements of adherence to QA and GLP were included.

Two females of the high-dose group died during the 2nd exposure, and one male of the high-dose group died after the 2nd exposure and another after the 17th exposure. In the low-dose group, one rat showed squatting position and piloerection on 2 days. In the mid-dose and high-dose rats, staggering gait, squatting position, tremors, hyperactivity, aggressiveness, tonoclonic convulsions and haematuria were observed. In addition, in the high-dose rats, salivation, contracted flanks, narrowed eye openings and piloerection were observed. There were no behavioural abnormalities in rats in any of the groups during the recovery period.

A statistically significant increase in body weight gain was noted in males (9%) and females (15%) of the high-dose group and for females of the mid-dose group (13%). In the high-dose group, a slight increase in feed consumption was found. Relative water consumption was increased during certain periods in mid- and high-dose rats. Haematology and clinical chemistry revealed no treatment-related effects. A few changes in relative organ weights were considered to be incidental and not related to treatment. No treatment-related macroscopic or microscopic findings were noted in the rats surviving the duration of the study. One male that died showed aspiration pneumonia. The other three animals that died showed cell atrophy in thymus and bone marrow and contraction of the spleen. In addition, blood congestion and focal or single hyperkeratosis in the forestomach were noted in two of these animals (one male and one female).

The no-observed-adverse-effect concentration (NOAEC) was 0.012 mg/l air, based on clinical signs of toxicity (neurotoxic symptoms) and increased body weight gain (females only) at 0.025 mg/l air (Hollander & Kramer, 1985).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a 2-year dietary carcinogenicity study, technical glufosinate-ammonium (purity 95.3%) was administered to groups of 60 male and 60 female NMRI mice at 0, 20, 80 or 160 ppm (equal to 0, 2.8, 10.8 and 23 mg/kg bw per day, respectively) for males and 0, 20, 80 or 320 ppm (equal to 0, 4.2, 16 and 64 mg/kg bw per day, respectively) for females. Ten mice of each sex per group were designated for interim sacrifice after 52 weeks of treatment. The mice were checked daily for mortality and clinical signs. A detailed clinical examination and palpation for nodules and masses were performed weekly. Feed consumption and body weight were recorded weekly during months 1–3 and biweekly thereafter. Ophthalmoscopy and hearing tests were performed on 10 animals of each sex per group pretest and at 6, 12, 18 and 24 months of treatment. Haematology (in five non-fasted mice of each sex per group) and clinical chemistry (in five fasted mice of each sex per group) were performed at weeks 52 and 104. Glutathione levels in whole blood and liver tissue were measured in the remaining fasted animals at termination of the study. Blood smears for differential counts were prepared from all surviving animals at termination of the study. At termination at 52 or 104 weeks of treatment, all animals were necropsied, organ weights were recorded and histological examinations were performed. Statements of adherence to QA and GLP were included.

At termination, increased mortality was observed in high-dose males (65%) compared with controls (43%). No effect of treatment on clinical signs, ophthalmoscopic and hearing examinations, haematology or feed consumption were noted. A reduction in body weight gain was observed in high-dose males of the interim kill group (up to 16%) during weeks 3–33 and in high-dose females of the terminal kill group (up to 9%) during weeks 7–31 of treatment. Isolated reductions in body weight gain were also observed in high-dose males of the terminal kill group. Clinical chemistry indicated an increased glucose level (138–167% of control) for high-dose male and female mice at 52 weeks of treatment, an increased AST activity (241% of control) for high-dose female mice at 52 weeks of treatment and a decreased glutathione level (58% of control) in whole blood for high-dose male mice at 104 weeks of treatment (not investigated in females after 104 weeks).

Furthermore, slightly decreased albumin (84% of control) and total protein levels (88% of control) were noted for high-dose female mice at 52 weeks of treatment, but not at 104 weeks. All other statistical differences in biochemical parameters were within the normal range for this mouse strain or lacked a dose–response relationship and were considered not to be treatment related. Absolute and relative liver weights were decreased (66–72% and 63–66% of controls, respectively) in females of all treatment groups, but not in males. As the reductions in liver weights in females were not observed in males and were not correlated to histopathological changes, they are considered not toxicologically relevant. Histopathological examination revealed no treatment-related changes in non-neoplastic or neoplastic lesions.

The NOAEL was 80 ppm (equal to 10.8 mg/kg bw per day), based on increased mortality and reduced body weight gain in males and changes in clinical chemistry parameters in both sexes at the next higher dose (males: 160 ppm, equal to 23 mg/kg bw per day; females: 320 ppm, equal to 64 mg/kg bw per day) (Suter, 1986a).

Rats

A 130-week combined toxicity and carcinogenicity study was performed according to OECD Test Guideline No. 453 in Wistar KFM-Han rats. The control group consisted of 130 rats of each sex, and treatment groups consisted of 80 rats of each sex per dose. Ten rats of each sex per group were killed after 52 weeks of treatment, and 20 rats of each sex per group were killed after 104 weeks. The groups of rats were fed diet with glufosinate-ammonium (purity 95.3%) at 0, 40, 140 or 500 ppm (equal to 0, 2.1, 7.6 and 26.7 mg/kg bw per day for males and 0, 2.5, 8.9 and 31.5 mg/kg bw per day for females of the chronic toxicity groups [104 weeks], respectively; and equal to 0, 1.9, 6.8 and 24.4 mg/kg bw per day for males and 0, 2.4, 8.2 and 28.7 mg/kg bw per day for females of the carcinogenicity groups [130 weeks], respectively). The rats were examined daily for mortality and clinical signs. Additionally, a detailed clinical examination was performed weekly. Body weight and feed consumption were recorded weekly for the first 3 months and every 2 months thereafter. Ophthalmoscopic evaluations were performed on 10 rats of each sex per group pretest and at 12, 24 and 30 months. Hearing tests were performed on 10 rats of each sex per group pretest and at 6, 12, 18, 24 and 30 months. Haematology, clinical chemistry and urine analysis were performed at weeks 26, 52, 78 and 104. Weights of adrenals, brain, gonads, heart, kidneys, liver, lung, pituitary, thymus and thyroid were determined at scheduled necropsy. Macroscopic and microscopic examinations of a wide range of organs and tissues were performed on all animals. Glutamine synthetase and ammonia levels were assessed in kidneys, liver and brain after 52 and 104 weeks. Ammonia levels were determined in urine and plasma after 26–28, 52–53, 78 and 104 weeks. Glutathione (reduced [GSH]/oxidized [GSSG]) levels were determined in liver and blood after 130 weeks of treatment. Liver (BSP) and kidney (PSP) function tests were performed on 10 rats of each sex of the control and high-dose groups after 43 and 96 weeks of treatment. Statements of adherence to QA and GLP were included.

There were no significant differences in mortality between treatment and control groups of the chronic toxicity animals for the major duration of the study. Only after 125 weeks was mortality in mid- and high-dose females (54% and 58% mortality, respectively) increased, compared with controls (30% mortality). No treatment-related clinical signs of toxicity were noted. In some treatment groups, statistically significant increases in body weight gain and feed consumption were observed during certain treatment periods. However, the increases were small (generally < 10%) and not considered

toxicologically significant. Similarly, increases observed in the absolute weights of some organs in these animals are also considered to be related to their increased body weight. It is concluded that organ weights were not affected by treatment. Eye and hearing examinations and urine analysis revealed no treatment-related findings. Slight reductions in haemoglobin concentrations and haematocrit in high-dose males and females and in erythrocyte counts in high-dose females were observed at week 52 only (Table 10). Mean corpuscular haemoglobin concentration was consistently increased in high-dose females at weeks 52, 78 and 104 (Table 11).

Table 10. Haematology after 52 weeks in rats administered glufosinate-ammonium

Parameter	Sex	Mean value			
		0 ppm	40 ppm	140 ppm	500 ppm
Red blood cells ($\times 10^{12}$/l)	Males	9.4	9.7	9.6	8.8
	Females	8.5	8.2	8.5	7.9*
Haemoglobin (mmol/l)	Males	9.3	9.5	9.3	8.7*
	Females	9.0	8.9	9.1	8.7*
Haematocrit (l/l)	Males	0.42	0.43	0.43	0.39*
	Females	0.42	0.40*	0.42	0.39*
Mean corpuscular haemoglobin concentration (mmol/l)	Males	22.0	22.0	21.9	22.3
	Females	21.5	22.1*	21.9	22.4*

From Suter (1986b)
* $P < 0.05$ (Dunnett test based on pooled variance or Steel test)

Table 11. Mean corpuscular haemoglobin concentrations after 52, 78 and 104 weeks in female rats administered glufosinate-ammonium

Week	Mean corpuscular haemoglobin concentration (mmol/l)			
	0 ppm	40 ppm	140 ppm	500 ppm
52	21.5	22.1*	21.9	22.4*
78	21.8	22.1	22.5*	22.4*
104	21.6	22.1*	22.0	22.3*

From Suter (1986b)
* $P < 0.05$ (Dunnett test based on pooled variance or Steel test)

No consistent effects on clinical chemistry parameters were observed. The data from the special biochemical investigations are presented in Tables 12 and 13. The biochemical studies indicate that, particularly in high-dose animals, glufosinate-ammonium treatment results in inhibition of glutamine synthetase activity in liver (up to 25%) and brain (up to 11% in high-dose females), decreases glutathione levels and increases the GSH to GSSG ratio in liver and blood. These effects are considered related to the structural analogies between glufosinate-ammonium and the substrate of glutamine synthetase (i.e. glutamate). The increased renal glutamine synthetase activity is considered to reflect adaptation to chronic treatment. In the absence of any related changes in organ function and histopathological changes, these biochemical changes are considered not toxicologically relevant. Ammonia levels were unaffected by treatment. Histological examination showed no effect of glufosinate-ammonium treatment on the incidences of non-neoplastic or neoplastic lesions.

Table 12. Glutamine synthetase activity after 52 and 104 weeks in the liver, kidney and brain of rats administered glufosinate-ammonium

Organ	Week	Sex	Mean glutamine synthetase activity (µmol/ml[a])			
			0 ppm	40 ppm	140 ppm	500 ppm
Liver	52	Males	2.28	2.07	1.80*	1.71*
		Females	2.85	2.67	2.38*	2.28*
	104	Males	2.33	2.39	2.21	2.35
		Females	2.92	3.28	3.56*	3.27
Kidney	52	Males	1.28	1.41	1.51*	1.72*
		Females	0.79	1.19*	1.27*	1.52*
	104	Males	1.47	2.03	2.15*	2.35*
		Females	1.19	1.61*	2.01*	2.28*
Brain	52	Males	2.48	2.48	2.52	2.34
		Females	2.35	2.37	2.19	2.14
	104	Males	ND	ND	2.34	2.19
		Females	2.53	2.32*	2.44	2.24*

From Suter (1986b)
ND, no data; * $P < 0.05$ (Dunnett test based on pooled variance or Steel test)
[a] Expressed in the table as micromoles of γ-glutamyl-hydroxamate formed per millilitre reaction mixture per 20 minutes at 37 °C.

Table 13. Glutathione (GSH, GSSG and total) concentration in the liver and whole blood after 130 weeks in rats administered glufosinate-ammonium

Organ	Sex	Glutathione	Mean glutathione concentration (µmol/g)			
			0 ppm	40 ppm	140 ppm	500 ppm
Liver	Males	GSH	5.49	5.37	5.59	6.02
		GSSG	1.53	1.43	1.19*	0.98*
		Total	7.02	6.80	6.78	6.99
	Females	GSH	4.98	4.50	3.10*	3.87*
		GSSG	1.32	1.39	1.26	1.30
		Total	6.30	5.89	4.36*	5.17*
Whole blood	Males	GSH	0.82	0.76	0.71	0.54*
		GSSG	0.20	0.19	0.24	0.32*
		Total	1.02	0.95	0.95	0.85
	Females	GSH	0.98	0.79	0.66*	0.64*
		GSSG	0.25	0.24	0.25	0.31
		Total	1.23	1.03	0.90*	0.95

From Suter (1986b)
* $P < 0.05$ (Dunnett test based on pooled variance or Steel test)

The NOAEL was 140 ppm (equal to 7.6 mg/kg bw per day), based on effects on haematology, GSH and GSSG levels and reduction of brain glutamine synthetase activity at 500 ppm (equal to 26.7 mg/kg bw per day) (Suter, 1986b).

In a 2-year carcinogenicity study performed in accordance with OECD Test Guideline No. 451, groups of 60 Wistar rats of each sex received diets containing glufosinate-ammonium (purity

96%) at a concentration of 0, 1000, 5000 or 10 000 ppm (equal to 0, 45, 229 and 466 mg/kg bw per day for males and 0, 57, 282 and 579 mg/kg bw per day for females, respectively). The animals were observed daily for mortality and clinical signs. A detailed clinical examination and palpation for nodules and masses were performed weekly. Body weight and feed consumption were recorded weekly for the first 13 weeks and biweekly thereafter. Blood samples for differential blood count were prepared from controls and animals at the high dose in weeks 52, 78 and 104. At termination, the animals were necropsied, and weights of adrenals, brain, gonads, heart, kidneys, liver, spleen, pituitary and thyroid were determined. Histopathological examinations of a wide range of organs and tissues were performed on all animals. Statements of adherence to QA and GLP were included.

No effects of treatment on mortality, clinical signs, the incidence of nodules or masses or differential blood count were observed. During the first 3–4 weeks, reductions in body weight gain were observed at 5000 ppm (up to 9% in weeks 2–3) and 10 000 ppm (up to 12% in week 2). After that period, no remarkable changes in body weight gain were noted. A dose-related increase in kidney weight of 15–30% was observed in all treatment groups. However, no histopathological changes were found in the kidneys. Necropsy showed a decreased incidence of pituitary nodules in males of all treatment groups and an increased incidence of adrenal gland foci in males at the high dose. Histopathology revealed an increase in the incidence of retinal atrophy in females at 5000 ppm (19/59) and in males and females at 10 000 ppm (12/60 and 29/59, respectively) compared with control males and females (4/60 and 3/58, respectively). No effect of glufosinate-ammonium treatment on the incidences of neoplasms was observed.

The NOAEL was 1000 ppm (equal to 45 mg/kg bw per day), on the basis of the increased incidence of retinal atrophy (Schmid et al., 1998).

2.4 Genotoxicity

Glufosinate-ammonium was tested for genotoxicity in a range of guideline-compliant assays. No evidence for genotoxicity was observed in any test.

The results of the genotoxicity tests are summarized in Table 14.

2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

In a range-finding one-generation reproductive toxicity study, glufosinate-ammonium (purity 95.3%) was administered in the diet during a 3-week premating period and continuing throughout the mating, gestation and lactation periods to groups of 10 Wistar/HAN rats of each sex per group. Dietary concentrations were 0, 50, 500, 2500 and 5000 ppm (equal to 0, 4.3, 44, 206 and 396 mg/kg bw per day for males and 0, 4.3, 44, 207 and 394 mg/kg bw per day for females, respectively, during the premating period; equal to 0, 3.2, 33, 163 and 327 mg/kg bw per day for males and 0, 4.7, 45, 205 and 407 mg/kg bw per day for females, respectively, during the gestation period). During the lactation period, females of the 50 and 500 ppm groups received 9.3 and 74 mg test material per kilogram body weight per day, respectively. The rats were checked daily for mortality and clinical signs. Body weight and feed consumption were measured weekly. In dams that littered, body weight and feed consumption were measured on lactation days (LDs) 1, 4, 7, 14 and 21. Litter size, sex of pups, number of stillbirths, number of live births and presence of gross anomalies were determined. Maternal rats were killed and necropsied at the end of lactation. The numbers of corpora lutea and implantation sites were recorded. Liver, spleen, kidneys and ovaries of all parental animals and two males and two females per litter were weighed. Statements of adherence to QA and GLP were included.

Table 14. Overview of genotoxicity tests with glufosinate-ammonium[a]

End-point	Test object	Concentration	Purity (%)	Results	Reference
In vitro					
Point mutations	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537; *Escherichia coli* WP2 *uvrA*	1.6–5000 µg/plate (±S9)	99.2	Negative	Ballantyne (2001a)[b,c]
Forward mutation	*Schizosaccharomyces pombe* strain SP ade 6-60/rad 10-1998, h-	125–1000 µg/ml (±S9)	95.3	Negative	Mellano (1984a)[b,d]
Mitotic recombination	*Saccharomyces cerevisiae* D4	1000–10 000 µg/ml (±S9)	95.3	Negative	Mellano (1984b)[b,e]
Chromosomal aberrations	Human lymphocytes	1000– 4640 µg/ml (−S9) 2150–10 000 µg/ml (+S9)	97	Negative	Mosesso (1990)[f]
Gene mutation	Mouse lymphoma L5178Y *Tk* locus	50–5000 µg/ml (−S9), 300–5000 µg/ml (+S9)	95.3	Negative	Cifone (1985)[g]
Gene mutation	V79 Chinese hamster cells, HPRT test	625–10 000 µg/ml (−S9) 625–8000 µg/ml (+S9)	97	Negative	Seeberg (1989)[h]
Unscheduled DNA synthesis	Male Fischer 344 rat hepatocytes	26.2–5240 µg/ml	95.3	Negative	Cifone (1984)[i]
In vivo					
Chromosomal aberrations	NMRI mouse bone marrow	100, 200 or 350 mg/kg bw (gavage)	96.9	Negative	Jung & Weigand (1986)[j]

DNA, deoxyribonucleic acid; S9, 9000 × *g* supernatant fraction of rat liver homogenate

[a] Positive and negative (solvent) controls were included in all studies.

[b] Statements of adherence to QA and GLP were included.

[c] Batch 26880-125-M29. Performed in accordance with OECD Test Guideline No. 471. Toxicity was observed at and above 200 µg/plate.

[d] Batch Lfd 12027.

[e] Batch Lfd 12027. Test design resembles OECD Test Guideline No. 481, with some minor deviations.

[f] Batch 27/85. Performed in accordance with OECD Test Guideline No. 473. In the absence of S9, marked cytotoxicity was observed at 10 000 µg/ml.

[g] Batch Lfd 12027. Study design resembles OECD Test Guideline No. 476.

[h] Batch 27/85. Performed in accordance with OECD Test Guideline No. 476. In the presence of S9, marked cytotoxicity was observed at concentrations of 5000 µg/ml and higher.

[i] Batch Lfd 12027. Study design resembles OECD Test Guideline No. 482. Cytotoxicity was observed at doses of 2620 µg/ml and higher.

[j] Glufosinate-ammonium (batch 13999) was administered by gavage to groups of NMRI mice (15 of each sex). The mice were killed at 24, 48 or 72 hours after administration of the test compound and negative control and at 24 hours after administration of the positive control. The frequencies of micronuclei in bone marrow polychromatic erythrocytes were determined. At 350 mg/kg bw, increased spontaneous activity, aggressiveness, tactile hyperaesthesia, motor excitation, uncoordinated gait, narrowed palpebral fissures and clonic convulsions were observed. At this dose, two females died. The study design generally resembles OECD Test Guideline No. 474; however, only 1000 immature erythrocytes per animal were scored for the incidence of micronuclei.

No mortality or clinical signs were observed. No significant reductions in body weight gain were observed during the premating period. During the first 2 weeks of the premating period, feed consumption was reduced by up to 19% in males at 2500 ppm (up to 14%) and 5000 ppm (up to 19%) and by up to 21% in females at 5000 ppm. During the lactation period, feed consumption was reduced by 22% in females of the 500 ppm group. This was considered to be related to the reduced number of pups per dam at this dose. No effects on mating performance or pregnancy rate were observed. Duration of pregnancy was not affected at 50 or 500 ppm. The dams at 2500 and 5000 ppm delivered no pups. At 500 ppm, the number of pups per litter (6.4) was markedly reduced compared with controls (12.7). No treatment-related postnatal loss of pups was found. Preimplantation loss was increased at 2500 and 5000 ppm, whereas post-implantation loss was increased at 500, 2500 and 5000 ppm. No effects of treatment on external malformations, sex ratio, development and behaviour of the offspring, body weight gain, feed consumption or organ weights were observed at 50 and 500 ppm. Necropsy revealed no treatment-related effect on parental rats.

Because of the total post-implantation loss observed in females at 2500 and 5000 ppm, a supplementary study was performed to clarify whether the effects on fertility were caused by the parent males or by the parent females. The males that had been treated continuously for 9 weeks at 0, 500, 2500 and 5000 ppm were mated with untreated mature females. No effect of treatment of males with glufosinate-ammonium at dietary concentrations up to 5000 ppm on mating performance, pregnancy rate, number of corpora lutea, rate of implantation and preimplantation and post-implantation loss were found.

The NOAEL for parental toxicity was 500 ppm (equal to 44 mg/kg bw per day), based on the reduced feed consumption in males at 2500 ppm (equal to 206 mg/kg bw per day). The NOAEL for offspring toxicity was 500 ppm (equal to 44 mg/kg bw per day, based on maternal intake), the highest dose at which dams produced a litter. With respect to reproduction, the NOAEL was 50 ppm (equal to 4.3 mg/kg bw per day), based on the reduced litter sizes (number of pups per dam) at 500 ppm (equal to 44 mg/kg bw per day) (Becker, 1986a).

In a two-generation dietary reproduction study, Wistar/Han rats (30 of each sex per group for the F_0 generation, 26 of each sex per group for the F_1 generation) were fed technical glufosinate-ammonium (purity 95.3%) at a dietary concentration of 0, 40, 120 or 360 ppm. The corresponding glufosinate-ammonium intakes during the different phases of the study are presented in Table 15.

The F_0 and F_1 generations were mated 2 times. Clinical examination of parental rats was performed daily and body weight was recorded weekly during the premating and gestation periods and on LDs 1, 4, 7, 14 and 21 (females). Feed consumption was recorded at the same time as the body weights, until day 14 postpartum. Rats were mated after 80 days of treatment (F_0, first mating) and about 10 days after weaning of the F_{1a} and F_{2a} generations (F_0 and F_1, second mating). Pups were weighed on LDs 1, 4, 7, 14 and 21. Twenty-six pups of the F_{1b} generation were selected for producing the F_{2a} and F_{2b} generations. F_{1b} rats were mated 101 days after weaning. All litters were examined (number of pups, sex of pups, number of stillbirths, number of live births, presence of gross anomalies). Necropsy was performed on F_0 rats, on one male and one female F_{1a} and F_{1b} pup per litter at LD 21 and on parental F_{1b} rats. The uterus was examined for metrial glands. In the control and high-dose groups, a full range of organs and tissues of the F_{1b} parental rats and one male and one female pup per litter of the F_{2b} generation was examined histologically, and selected organs from these rats were weighed. Statements of adherence to QA and GLP were included.

No effect of treatment was observed on viability, behaviour, clinical signs and general appearance, body weight and body weight gain, precoital time, pregnancy rate, duration of gestation, fertility, parturition, lactation or nursing. A significantly reduced feed consumption (up to 20%) was noted in the F_0 and F_1 parent females in the 360 ppm group during the lactation periods for breeding the F_{1a}, F_{1b}, F_{2a} and F_{2b} litters. This is considered to be related to the smaller litter size at this dose. No effect was seen at other time periods at this dose level or at 40 or 120 ppm. No effects were seen in males. At 360 ppm, litter size was significantly reduced (average of F_{1a}, F_{1b}, F_{2a} and F_{2b} is 8.5

pups/litter versus 11.2 pups/litter in controls). This finding corresponds to the result generated in the preliminary study. The decrease in pup number per litter in the high-dose group is considered by the study authors to reflect an interference during the period of implantation due to a toxic effect on early embryonic development. Non-dose-dependent increases in absolute (up to 14%) and relative (up to 15%) kidney weights were found at 120 ppm in male rats and at 360 ppm in male and female rats. The increases in kidney weights were not accompanied by gross pathological or histopathological changes and were therefore considered not to be adverse. Similar changes were also regularly observed in subchronic toxicity studies and should be considered as related to glufosinate-ammonium administration. No treatment-related changes were found in the F_{1a}, F_{1b}, F_{2a} and F_{2b} pups with respect to viability, postnatal loss, breeding loss, rate of malformations and/or anomalies, necropsy findings and organ weights. Macroscopic and histopathological examinations of the F_{1b} and F_{2b} generations revealed no effect of treatment.

Table 15. Glufosinate-ammonium intake in parental rats during different phases of the multigeneration reproductive toxicity study

Generation	Sex	Intake (mg/kg bw per day)		
		40 ppm	120 ppm	360 ppm
F_0 premating	Male	3.3	10	30
F_0 post-mating—F_{1a}	Male	2.0	6.8	20
F_0 post-mating—F_{1b}	Male	2.0	6.2	19
F_0 premating	Female	3.6	11	33
F_0 gestation—F_{1a}	Female	3.0	9.3	28
F_0 lactation—F_{1a}	Female	6.3	19	51
F_0 gestation—F_{1b}	Female	3.3	9.7	30
F_0 lactation—F_{1b}	Female	6.3	20	49
F_1 premating	Male	3.0	9.4	28
F_1 post-mating—F_{2a}	Male	2.0	6.7	19
F_1 post-mating—F_{2b}	Male	2.0	6.0	18
F_1 premating	Female	3.5	10	31
F_1 gestation—F_{2a}	Female	3.0	8.7	27
F_1 lactation—F_{2a}	Female	6.3	18	47
F_1 gestation—F_{2b}	Female	3.0	8.7	25
F_1 lactation—F_{2b}	Female	5.7	19	45

From Becker (1986b)

The NOAEL for parental toxicity was 360 ppm (equal to 18 mg/kg bw per day), the highest dose tested. The NOAEL for offspring toxicity was 360 ppm (equal to 18 mg/kg bw per day), the highest dose tested. With respect to reproduction, the NOAEL was 120 ppm (equal to 8.7 mg/kg bw per day, based on maternal intake during gestation), based on the reduced litter sizes (number of pups per dam) noted in all litters of both generations at 360 ppm (equal to 18 mg/kg bw per day) (Becker, 1986b).

(b) Developmental toxicity

Rats

In a developmental toxicity study, groups of 20 mated female Wistar rats were treated orally, by gavage, with glufosinate-ammonium (purity 97.7%) in distilled water at a dose level of 0, 10, 50 or 250 mg/kg bw per day from days 7 through 16 of gestation (day 1 = day on which sperm were

detected in the vaginal smear). The doses were based on the results of a range-finding study. Clinical signs and feed consumption were recorded daily. Body weight was measured weekly. Feed consumption was measured over 2-day periods. All females were killed on day 21 of gestation. The uterus was examined, and the numbers of live and dead fetuses, corpora lutea and implantations were counted. Body weight, crown–rump lengths and sex ratios of the fetuses as well as placental weight and diameter of fetuses undergoing resorption were recorded. About half of the fetuses from each litter were selected for skeletal examinations, and the other half for cross-sectional visceral examinations. Statements of adherence to QA and GLP were included.

Clinical signs, such as motorial unrest, hyperactivity, piloerection, flabbiness, squatting and arching of the spine, were noted in dams at 50 and 250 mg/kg bw per day. The clinical signs started between days 9 and 11 and persisted for 3–5 days. Two treated dams from the 10 mg/kg bw per day group also presented signs of hyperactivity. However, this finding was not clearly treatment related and could not be repeated in a supplementary study. Therefore, it was considered not to be significant. Feed consumption was significantly reduced (19%) during days 14–17 of treatment in the 250 mg/kg bw per day group. Body weight gain was slightly reduced (up to 6% at GD 17) at 250 mg/kg bw per day. Feed consumption and body weight gain were not affected at 10 or 50 mg/kg bw per day.

All dams in the 10 mg/kg bw per day and control groups, 16 dams in the 50 mg/kg bw per day group and 10 dams in the 250 mg/kg bw per day group carried live fetuses to full term. Macroscopic examination of dams killed at GD 21 and their fetuses revealed no treatment-related effects. At the high dose, eight dams were killed following vaginal haemorrhage, first observed after four treatments, and one dam died on study day 17. At 50 mg/kg bw per day, four dams were killed following vaginal haemorrhage, first observed after three treatments. The vaginal haemorrhages probably are the result of abortions, as indicated by vacant implantation sites and the presence of conceptuses in the birth canal of some dams. The numbers of corpora lutea and implantations were not affected by treatment. The litters of the dams that carried live fetuses to full term were of the same sizes as those in the control group. The live fetuses in these litters in the treated groups were normally developed, with normal body weights and body lengths, and the male/female ratio was balanced.

In the dams that were sacrificed prematurely in the 50 and 250 mg/kg bw per day groups as a result of vaginal haemorrhage or dams that had only dead implantations in the uterus, relatively small spleens (0.1–0.17 g versus 0.61 ± 0.1 g in controls) and relatively large adrenals (0.1–0.14 g versus 0.072 ± 0.009 g in controls) were frequently observed.

The dam in the 250 mg/kg bw per day group that died on day 17 had 13 normally developed conceptuses. In the dams that were killed due to vaginal haemorrhages, the following uterine findings were recorded. Three early implantations in the birth canal were found in one animal of the 50 mg/kg bw per day group and one in an animal from the 250 mg/kg bw per day group. There were nine other normally developed early implantations in the uterus of each of these animals. One animal in the 50 mg/kg bw per day group and three in the 250 mg/kg bw per day group had only implantation sites without embryonic tissue. In one dam from the 50 mg/kg bw per day group and three dams from the 250 mg/kg bw per day group, only embryonic resorptions were found. One dam in the 50 mg/kg bw per day group, which was killed on day 20 of gestation, had only stunted, live fetuses with weights up to 1.15 g (premature delivery) and a supernumerary implantation site without embryonic tissue. One dam in the 250 mg/kg bw per day group, which was killed on day 19 of gestation, showed stunted fetuses, both live and dead, with weights up to 0.62 g. No remnants of aborted fetuses from any of the dams with vaginal haemorrhages were found in the cage litter. One dam in the 250 mg/kg bw per day group without vaginal haemorrhages delivered only stunted, dead fetuses and a supernumerary implantation site without fetal tissue on day 21 of gestation.

The placentas of the live fetuses showed no macroscopic abnormalities, and their weights were within the range of previous control values. The placentas of the dead fetuses from the dam in the 250 mg/kg bw per day group were smaller than those of the live fetuses in the other litters. No malformations were observed in any of the fetuses.

In the fetuses, a dose-related incidence of distension of the renal pelvis and/or ureter was found in all treatment groups (10%, 18.5%, 25.3% and 31.6% at 0, 10, 50 and 250 mg/kg bw per day, respectively), reaching statistical significance at the high dose. The maximum incidence of historical controls was 13.2%. A slight retardation of skeletal ossification of os metacarpale 5 was observed in the 250 mg/kg bw per day group. Incidences of other variations were not related to treatment.

The NOAEL for maternal toxicity was 10 mg/kg bw per day, based on clinical signs and abortions observed at 50 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 10 mg/kg bw per day, based on intrauterine deaths and increased incidence of distension of the renal pelvis and/or ureter at 50 mg/kg bw per day. No evidence of a teratogenic effect was observed (Baeder, Weigand & Kramer, 1985a).

In a developmental toxicity study, groups of 20 mated female Wistar rats were treated orally, by gavage, with glufosinate-ammonium (purity 97.2%) in distilled water at a dose level of 0, 0.5, 2.24 or 10 mg/kg bw per day from days 7 through 16 of gestation (day 1 = day on which sperm were detected in the vaginal smear). Clinical signs and feed consumption were recorded daily. Body weight and feed consumption were measured on days 0, 7, 14, 17 and 21. All females were killed on day 21 of gestation. All dams were necropsied and examined macroscopically for abnormalities, and selected organs were weighed. The uterus was examined, and the numbers of live and dead fetuses, corpora lutea and implantations were counted. Body weight, crown–rump lengths and sex ratios of the fetuses as well as placental weight and diameter of fetuses undergoing resorption were recorded. About half of the fetuses from each litter were selected for skeletal examinations, and the other half for cross-sectional visceral examinations. Statements of adherence to QA and GLP were included.

No mortality or clinical signs were observed. The feed consumption and body weight gains of the dams were not affected by treatment. At terminal necropsy, no gross findings or organ weight changes were seen in the treated groups. All dams in all groups carried live fetuses to full term. Treatment had no effect on the numbers of corpora lutea, implantations, embryonic resorptions or live and dead fetuses per dam, fetal development, body weights, body lengths or sex ratio. The placentas of the fetuses showed no macroscopic abnormalities, and they were normal in weight. No effect of treatment on external anomalies or anomalies of the internal organs and skeleton was revealed.

The NOAEL for both maternal and fetal toxicity was 10 mg/kg bw per day, the highest dose tested (Baeder, Weigand & Kramer, 1985b; Hoerlein & Gorbach, 1985).

In a developmental toxicity study, groups of 20 mated female Wistar rats were treated orally, by gavage, with glufosinate-ammonium (purity 97.2%) in distilled water at a dose level of 0, 0.5, 2.24 or 10 mg/kg bw per day from days 7 through 16 of gestation (day 1 = day on which sperm were detected in the vaginal smear). Clinical signs and feed consumption were recorded daily. Body weight and feed consumption were measured on days 0, 7, 14, 17 and 21. All dams were allowed to deliver normally and to rear their offspring for 21 days (up to weaning). During the 21-day lactation period, body weights of the dams were recorded on days 0, 7, 14 and 21 postpartum. The offspring were examined daily for viability and general behaviour, and their body weights were recorded on days 4, 7, 14 and 21 after delivery. Their physical development was evaluated by recording the time of pinna separation, start of coat growth, incisor eruption and eyelid opening. Dams and offspring were killed between days 21 and 23 postpartum, dissected and examined macroscopically. Selected organs were weighed. Offspring with externally visible anomalies were stained and examined for skeletal anomalies. Statements of adherence to QA and GLP were included.

One dam in each of the 0.50 and 2.24 mg/kg bw per day groups had not littered by GD 25 and were killed. Examination of these females revealed that one dam had only empty implantation sites and the other dam had only two implantation sites and one dead, normally developed fetus. No mortality or clinical signs were observed. The feed consumption, body weight gains and gestation duration of the dams were not affected by treatment. Treatment did not affect the number of live offspring per litter, sex ratio, body weight at birth, offspring mortality, physical development and

body weight gain, general behaviour of the offspring and survival rate of the offspring at 21 days. At necropsy, no treatment-related external anomalies or organ weight changes were observed in dams and offspring.

The NOAEL for maternal and offspring toxicity was 10 mg/kg bw per day, the highest dose tested (Pensler et al., 1986).

Rabbits

In a developmental toxicity study, groups of 15 pregnant Himalayan rabbits were treated orally, by gavage, with glufosinate-ammonium (purity 95.3%) in distilled water at a dose level of 0, 2, 6.3 or 20 mg/kg bw per day from days 7 through 19 of gestation (day 0 = day of mating). The doses were based on the results of a range-finding study. Clinical signs were recorded daily. Body weight and feed consumption were measured weekly for the first 3 weeks and once again 9 days later. All females were killed on day 29 of gestation. All does were necropsied and examined macroscopically for abnormalities, and selected organs were weighed. The uterus was examined, the numbers of live and dead fetuses, corpora lutea and implantations were counted, and placental weight and diameter of fetuses undergoing resorption were recorded. The fetuses were weighed and reared for 24 hours in an incubator to assess viability. After this, the crown–rump lengths and sex ratios were recorded. About half of the fetuses from each litter were selected for skeletal examinations, and the other half for cross-sectional visceral examinations. Statements of adherence to QA and GLP were included.

At the high dose, a 115 g reduction in body weight was observed during the 1st week of treatment (GDs 7–14), while the control does gained 51 g. The mid-dose females gained 1 g during this period (not statistically significantly different from controls). Body weight gains of all groups were similar from GD 14 onwards. At termination, the body weight of high-dose rabbits was 94% of that of controls. During the 1st week of treatment, feed consumption was reduced at the middle and high doses (2.06 and 1.28 g/100 g bw, respectively) compared with controls (3.25 g/100 g bw). During the 2nd week of treatment, reduced feed consumption was observed only at the high dose (1.82 g/100 g bw versus 2.96 g/100 g bw in controls). A reduction in the quantity of faeces at the high dose is considered to be related to the decreased feed consumption.

A slightly higher kidney weight (11%) was found at 20 mg/kg bw per day. Three does at the high dose were killed prematurely: one doe displaying severe clinical signs was killed at GD 17 for humane reasons (necropsy revealed seven conceptuses and a resorption site), one doe aborted on GD 20 (necropsy revealed six live fetuses and one dead fetus) and one doe delivered prematurely on GD 25 (six dead fetuses and placentas were found). At termination, one doe at 20 mg/kg bw per day had seven implantation sites and two conceptuses under resorption in the uterus, but no live fetuses. At 6.3 mg/kg bw per day, one doe died on GD 29 while giving premature birth (necropsy revealed five placentas and five living and two dead fetuses). The numbers of abortions and premature deliveries are within the historical control range. All does in the control and 2 mg/kg bw per day groups carried live fetuses to full term. There was no difference in the numbers of corpora lutea and implantations or in the litter sizes and sex ratios in the treatment groups compared with the controls. The live fetuses delivered on day 29 of gestation in the treated groups were normally developed, with normal body weights and body lengths. The aborted and prematurely delivered fetuses, as well as those from the doe that was killed intercurrently, were normally developed for the stage at which gestation had been interrupted. The placentas of the dead fetuses were in most cases smaller than those of the live fetuses and were also anaemic.

No effect of treatment on the external development or internal organs and skeleton was revealed.

The NOAEL for maternal toxicity was 6.3 mg/kg bw per day, on the basis of clinical signs, body weight loss and reduced feed consumption, increased number of abortions and dead fetuses and increased kidney weight at 20 mg/kg bw per day. The NOAEL for fetal toxicity was 6.3 mg/kg bw per day, on the basis of an increased number of dead fetuses at 20 mg/kg bw per day (Baeder, Weigand and Kramer, 1984; Baeder, Mayer & Langer, 1986; Debruyne, 2003).

2.6 *Special studies*

(a) Neurotoxicity

Mice and rats

A single dose of glufosinate-ammonium (purity 95.3%) was administered by gavage to groups of five female NMRI mice and five female Wistar rats. Mice received 0, 50 or 200 mg/kg bw, whereas rats were dosed with 0, 200 or 800 mg/kg bw. Four hours after treatment, the animals were killed, and glutamine synthetase activity and the ammonium level were determined in brain, liver, kidneys and heart. In the rat, the glutamine and glutamic acid levels in these organs were also determined. Statements of adherence to QA and GLP were included.

No clinical signs were present during the 4-hour observation period. The results of glutamine synthetase activity measurements in the brain, liver, kidneys and heart of rats and mice 4 hours after oral administration are presented in Table 16.

Table 16. Effect of glufosinate-ammonium on glutamine synthetase activity in mice and rats

Dose (mg/kg bw)	Glutamine synthetase activity (± standard deviation)[a]			
	Brain	Liver	Kidneys	Heart
Female mice				
0	36.2 (± 0.8)	32.2 (± 1.1)	16.5 (± 0.7)	0.8 (± 0.2)
50	35.0 (± 0.6)	31.5 (± 1.6)	8.2* (± 0.3)	1.4* (± 0.1)
200	34.2 (± 0.9)	30.9 (± 0.8)	6.0* (± 1.2)	1.3 (± 0.2)
Female rats				
0	13.9 (± 0.3)	9.3 (± 0.7)	2.5 (± 0.1)	0.4 (± 0.02)
200	13.8 (± 0.4)	6.8 (± 1.1)	0.8* (± 0.1)	0.5 (± 0.05)
800	14.6 (± 0.2)	2.8* (± 0.8)	1.0* (± 0.2)	0.5 (± 0.04)

From Ebert & Kramer (1985b)
* $P < 0.05$ (according to the Dunnett test)
[a] Expressed in the table as micromoles of γ-glutamyl-hydroxamate formed per millilitre reaction mixture per 20 minutes at 37 °C.

Glufosinate-ammonium had no effect on glutamine synthetase activity in either brain or heart, even at high doses. Glutamine synthetase activities in the kidneys of both species and in the rat liver were markedly reduced compared with the corresponding control values.

No change in ammonium levels was observed in the brain, kidneys or heart of treated rats or mice. In the liver, a slight (14%) but statistically significant increase in ammonium level was detected only in mice at 200 mg/kg bw. Glutamine and glutamic acid levels in rat brain, liver, kidneys and heart showed treatment-related changes (Ebert & Kramer, 1985b).

Rats

In an acute oral neurotoxicity study, Wistar rats (10 of each sex per dose) were treated by gavage with glufosinate-ammonium 50% aqueous technical concentrate (purity 50.2%) in bi-distilled water at a dose of 0, 10, 100 or 500 mg/kg bw. Mortality and clinical signs were observed daily up to 15 days post-dosing. Feed consumption and body weights were recorded pretest and weekly during the observation period. Functional observational battery, locomotor activity, body temperature, rearing, landing foot splay and grip strength were measured pretest and at 1, 7 and 14 days after application. All animals were killed on test day 15 and examined macroscopically. Statements of adherence to QA and GLP were included.

No mortality was observed. Hunched posture, tachypnoea, ruffled fur and emaciation were observed in one high-dose female on the 2nd and 3rd days after treatment. This animal showed ptosis, tachypnoea, hunched posture and piloerection in the functional observational battery test 1 day after application. No other treatment-related findings were noted during the functional observational battery tests. Feed consumption, body weights, locomotor activity, grip strength, body temperature, rearing and landing foot splay were unaffected by treatment.

The NOAEL was 100 mg/kg bw, based on clinical signs observed in the home cage and in the functional observational battery in one animal at 500 mg/kg bw (Hamann, 1999a).

In an acute oral neurotoxicity study, Wistar rats (10 of each sex per dose) were treated by gavage with glufosinate-ammonium 50% aqueous technical concentrate (purity 50.2%) in bi-distilled water at a dose of 0, 10, 100 or 500 mg/kg bw. Mortality and clinical signs were observed daily up to 15 days post-dosing. Feed consumption and body weights were recorded pretest and weekly during the observation period. Water maze tests evaluating learning, memory and relearning skills were performed with all animals pretest and at 1, 7 and 14 days after application. All animals were killed on test day 15 and examined macroscopically. Histological examinations were performed on the brain, the spinal cord and the sciatic tibial nerves from all animals after perfusion fixation. Statements of adherence to QA and GLP were included.

No mortality or clinical signs were noted. Body weight and feed consumption were not affected by treatment. No treatment-related neurotoxic effects were observed with the water maze test. Macroscopic and histopathological examinations revealed no effect of treatment with glufosinate-ammonium.

The NOAEL was 500 mg/kg bw, the highest dose tested (Hamann, 1999b).

Groups of 10 male and 10 female Wistar rats were given glufosinate-ammonium aqueous technical concentrate (purity 50.2%) at a dietary level of 0, 20, 200 or 2000 ppm (equal to 0, 1.5, 15 and 143 mg/kg bw per day for males and 0, 1.8, 17 and 162 mg/kg bw per day for females, respectively) for up to 38 days. An additional five rats of each sex were used at each dose level for determining glutamine synthetase activity in the liver, kidney and brain at the end of the treatment period. The animals were observed daily for clinical signs, whereas body weight and feed consumption were recorded weekly. A functional observational battery, locomotor activity, body temperature, landing foot splay distance and grip strength were measured pretest and after 2 and 4 weeks of treatment. A water maze test was performed pretest and in week 5. A single dose of midazolam (2 mg/kg bw) given intraperitoneally before or 10 minutes after the water maze test was used as a positive control. At termination, all the animals were killed and examined macroscopically. The brain, heart, liver and kidneys were weighed, and histopathological examination was performed on the brain, spinal cord, and sciatic and tibial nerves of all the control and high-dose animals. Statements of adherence to QA and GLP were included.

No treatment-related mortality or clinical signs were observed. Body weight gain, feed consumption, functional observational battery parameters, locomotor activity, rearing, grip strength, body temperature, landing foot splay, or learning and memory tests (water maze) were unaffected by treatment with glufosinate-ammonium.

In animals receiving glufosinate-ammonium, a statistically significant dose-related inhibition of glutamine synthetase activity was observed in the liver of both sexes and the kidney of males only at all dose levels. Inhibition of glutamine synthetase activity was also observed in the brain tissue of males at 200 ppm and in both sexes at 2000 ppm (see Table 17). A greater than 50% reduction of glutamine synthetase activity in the liver and/or a greater than 10% reduction of glutamine synthetase activity in the brain are considered to be adverse. In the absence of histopathological findings in the kidney, the reduction in glutamine synthetase activity was considered to be a non-adverse finding.

Table 17. Mean glutamine synthetase activity following 38 days of treatment with glufosinate-ammonium in rats

Organ	% of control activity					
	Males			Females		
	20 ppm	200 ppm	2000 ppm	20 ppm	200 ppm	2000 ppm
Liver	67**	44**	35**	77*	55**	37**
Kidney	75**	64**	65**	98	93	94
Brain	96	93*	75**	102	100	73**

From Hamann et al. (2000)
* $P < 0.05$; ** $P < 0.01$ (according to the parametric method of Dunnett)

Relative kidney weight was 15% increased in high-dose males. No treatment-related macroscopic or microscopic abnormalities were seen in any of the animals.

The NOAEL for glufosinate-ammonium was 20 ppm (equal to 1.5 mg/kg bw per day), based on a greater than 50% reduction in glutamine synthetase activity in the liver in males at 200 ppm (equal to 15 mg/kg bw per day) (Hamann et al., 2000).

The effect of subchronic treatment with glufosinate-ammonium on the activity of glutamine synthetase was investigated in liver, kidneys and brain. Glufosinate-ammonium (supplied as an aqueous technical concentrate; concentration 50.2% weight per weight [w/w]) was administered in the diet to groups of 10 male Wistar rats for 6, 13, 20 or 90 days at a dose of 100 or 1000 ppm. Additional groups of 10 male Wistar rats received glufosinate ammonium at a dose of 100 or 1000 ppm for 91 days followed by a 30-day recovery period. Control groups of 10 male Wistar rats received the untreated diet for the same period of time. Achieved nominal intakes of glufosinate-ammonium were 6.2 and 63.6 mg/kg bw per day at 100 and 1000 ppm, respectively. Mortality and clinical signs were recorded daily, and feed consumption and body weights were measured weekly. All animals were necropsied at scheduled sacrifice. Organ weights (brain, liver and kidney) were measured, and all macroscopic findings were recorded. Samples of liver, kidney and brain were collected from all animals and processed for measurement of glutamine synthetase activity. Statements of adherence to QA and GLP were included.

No treatment-related effects were noted for clinical signs, feed consumption or body weight.

Glufosinate-ammonium induced a significant inhibition of glutamine synthetase activity in the liver and kidney (see Table 18). In the brain, only a slight inhibition was observed at the high dose of glufosinate-ammonium. No cumulative effect over time was observed. The effects were (almost) completely reversed after a 30-day recovery period.

There were no treatment-related macroscopic findings or effects on liver and brain weights. Necropsy at 6, 13 or 20 days showed an increase in absolute and relative kidney weights in rats treated with glufosinate-ammonium (up to 23%) that was fully reversible after 30 days of recovery.

The NOAEL for glufosinate-ammonium was 100 ppm (equal to 6.2 mg/kg bw per day), based on a greater than 50% reduction in glutamine synthetase activity in the liver and a greater than 10% reduction in glutamine synthetase activity in the brain at 1000 ppm (equal to 63.6 mg/kg bw per day) (Schmid et al., 1999).

Table 18. Inhibition of glutamine synthetase activity in the rat by glufosinate-ammonium

Tissue	Sacrifice after day:	% inhibition	
		100 ppm	1000 ppm
Liver	6	45**	64**
	13	49**	70**
	20	49**	67**
	90	40**	60**
	Recovery	3	15*
Brain	6	0	11**
	13	0	9**
	20	0	15**
	90	0	18**
	Recovery	2	12**
Kidney	6	40**	42**
	13	39**	42**
	20	38**	47**
	90	33**	54**
	Recovery	10	3

From Schmid et al. (1999)
* $P < 0.05$; ** $P < 0.01$ (according to the Dunnett test based on pooled variance)

In a dietary developmental neurotoxicity study, glufosinate-ammonium (purity 50.8%) was administered to 25 mated female Crl:C (SD) IGS BR rats at 0, 200, 1000 or 4500 ppm (equal to 0, 14, 69 and 292 mg/kg bw per day during gestation and 0, 36, 176 and 756 mg/kg bw per day during lactation, respectively). All animals were observed daily for mortality and clinical signs. A detailed clinical examination of the dams was performed on GDs 6 and 13 and on postnatal days (PNDs) 10 and 21. Body weights and feed consumption were recorded daily during the treatment period. After parturition, the numbers of dead and live pups were recorded, and the pups were sexed and examined for malformations. Females that did not deliver or with total litter loss were killed and examined macroscopically. On PND 4, litters were culled to 10 pups per litter. Females with litters that consisted of fewer than eight pups or fewer than four pups of each sex were killed on LD 5 and examined macroscopically. All remaining females with viable pups were killed after PND 21 and examined macroscopically. Litters were examined daily for clinical signs. On PNDs 4, 11 and 21 and weekly thereafter, a detailed physical examination of the pups was performed until termination on PND 72. The 1st day of balanopreputial separation and vaginal patency was recorded for all males and females, respectively. Twenty selected pups of each sex per group (subset A) were subjected to a detailed clinical examination on PNDs 4, 11, 21, 35, 45 and 60, acoustic startle response test on PNDs 20 and 60, locomotor activity tests on PNDs 13, 17, 21 and 61 and learning and memory test (water maze) on PND 62. From this subset, 10 pups of each sex per group were selected for neuropathological, morphometric and brain weight examinations on PND 72. A second subset (B) of 20 pups of each sex per group was subjected to the learning and memory test on PND 22. A third subset (C) of 10 pups of each sex per group was selected for neuropathological, morphometric and brain weight examinations on PND 21. All non-selected pups were killed and examined macroscopically on PND 28. Statements of adherence to QA and GLP were included.

No mortality was observed. In the high-dose group, light-coloured faeces were observed in dams primarily between GD 8 and GD 13. In this group, statistically significant reductions in mean maternal body weight gains (8% on GD 20) and feed consumption (17% between GD 6 and GD 20) were noted. On the 1st day of treatment, all treatment groups showed a dose-dependent body weight

loss (1–6 g), while control animals gained 4 g. The reduction in body weight gain in the low- and mid-dose groups was observed during the first 4–5 days of treatment. Feed consumption was also dose-dependently decreased in the low- and mid-dose groups during the first 4–5 days of treatment and in the high-dose dams throughout the treatment period. The initial body weight and feed consumption effects were attributed to the decreased palatability of the test diet at the initiation of the diet administration and are not considered to be a systemic effect of glufosinate-ammonium. No treatment-related clinical observations were noted for dams on GDs 6 and 13 and PNDs 10 and 21. Mean gestation length, gestation index, mean number of pups born, mean live litter size and sex ratio per litter were unaffected by glufosinate-ammonium treatment. At scheduled termination, necropsy of the dams revealed no effects of treatment. The mean number of implantation sites, number of pups born and numbers of unaccounted sites recorded at scheduled necropsy were unaffected by treatment. No macroscopic findings were noted at the scheduled necropsy of F_0 females on LD 21.

No clinical signs of toxicity were observed in treated pups. Slight, but non-statistically significant, reductions in mean body weights of male and female offspring (7% and 6% for males and females, respectively) were noted in the 4500 ppm group on PND 1. Body weights were statistically significantly reduced by 18–19% on PND 21 at this dose. At 1000 ppm, body weights were about 9–10% lower than those of controls during the period up to weaning, occasionally reaching statistical significance. During the period up to PND 70, body weights in the high-dose group were 6–9% lower than those of controls. Total motor activity was increased in high-dose pups at PND 17 and in both sexes of the mid- and high-dose groups at PND 21. At PND 61, total motor activity of males and females was slightly increased at 1000 and 4500 ppm, either during part of the 60-minute locomotor activity test or over the total duration of the test. Patterns of habituation were comparable between treatment and control groups. No treatment-related effects were observed on acoustic startle or learning and memory tests. Histological examination of the brains of pups revealed a significant decrease in vertical height between the layers of pyramidal neurons in the hippocampal formation in males (11%) and in radial thickness of the cortex in females (8%) of the high-dose group. The decrease (7%) in vertical height between the layers of pyramidal neurons in the hippocampal formation in mid-dose males was not statistically significant.

The NOAEL for maternal toxicity was 1000 ppm (equal to 69 mg/kg bw per day), based on decreased body weight gain and feed consumption at 4500 ppm (equal to 292 mg/kg bw per day). The NOAEL for offspring toxicity was 200 ppm (equal to 14 mg/kg bw per day), based on reduced body weight gain during the preweaning period, effects on motor activity at PNDs 17, 21 and 62 and the decrease in vertical height between the layers of pyramidal neurons in the hippocampal formation in males at 1000 ppm (equal to 69 mg/kg bw per day) (Nemec, 2004).

Glufosinate-ammonium was administered once orally by gavage to groups of 5–10 female Wistar rats at 0, 200, 800 or 1600 mg/kg bw. After treatment, mortality rates and clinical signs were recorded for 7.5 days. The animals were also subjected regularly to a comprehensive observational assessment. The surviving animals were weighed and subsequently killed and dissected 24 hours or 3 or 7.5 days after dosing. Glutamine synthetase activity and glutamate and ammonium levels were measured in the brain, liver and kidneys. Acetylcholinesterase activity was also measured in the brain. Statements of adherence to QA and GLP were included.

In the high-dose group (1600 mg/kg bw), three animals died. Clinical signs in the 200 mg/kg bw group consisted of enhanced spontaneous activity and isolated piloerection on day 1 after treatment. At 800 mg/kg bw, frequent enhancement of spontaneous activity and, in one case, convulsions and Straub tail from 12 to 24 hours after treatment were observed. At 1600 mg/kg bw, diarrhoea, convulsions, enhancement of spontaneous activity and piloerection were observed, starting 6 hours after treatment. Additionally, 2–3 days after treatment, tonoclonic convulsions, squatting position, contracted flanks, lagophthalmos, drowsiness, reduced respiratory rate and blood-encrusted eyelids and mouth were observed. The comprehensive observational assessments of the animals in the 200 and 800 mg/kg bw groups revealed marginal impairment of behaviour in the form of reduced motivation and performance, which began to recede quite noticeably from day 5 onwards. In the high-

dose group, a tendency to convulsions and spasms followed by exhaustion was observed, starting 1.5 days after treatment All signs of intoxication began to disappear 3.5 days after treatment.

The effect of glufosinate-ammonium on organ glutamine synthetase activity is presented in Table 19.

Table 19. Effect of glufosinate-ammonium on glutamine synthetase activity in various organs

Dose (mg/kg bw)	Glutamine synthetase activity					
	1 day after treatment		3 days after treatment		7.5 days after treatment	
	Mean (± SD) (mg/g tissue in 20 min)	% change relative to control	Mean (± SD) (mg/g tissue in 20 min)	% change relative to control	Mean (± SD) (mg/g tissue in 20 min)	% change relative to control
Brain						
0	65 (± 4.1)	—	51.5 (± 2.9)	—	45 (± 2.6)	—
200	61 (± 9.8)	−6	49 (± 1.6)	−5	43.5 (± 5.3)	−3
800	56 (± 6.3)	−14	46 (± 3.3)	−11	46 (± 1.4)	−12
1600	44* (± 5.4)	−32	26.5^{+} (± 10.8)	−49	32^{+} (± 4.3)	−29
Liver						
0	38 (± 6.3)	—	25.5 (± 3.0)	—	52 (± 9.0)	—
200	10* (± 1.1)	−74	13.5* (± 1.5)	−47	29* (± 2.9)	−44
800	10.5* (± 1.7)	−72	9* (± 1.9)	−65	29* (± 4.4)	−44
1600	8* (± 1.2)	−79	7* (± 2.0)	−73	25* (± 2.6)	−52
Kidneys						
0	17 (± 1.5)	—	23 (± 0.7)	—	15 (± 1.5)	—
200	8* (± 1.2)	−53	13 (± 0.8)	−43	14 (± 0.4)	−7
800	6* (± 0.6)	−65	10.5^{+} (± 0.8)	−54	14 (± 1.3)	−7
1600	4.5* (± 0.4)	−74	9.5^{+} (± 1.0)	−59	16 (± 1.1)	+5

From Ebert et al. (1986a)
SD, standard deviation; * $P < 0.05$ (procedure of Dunnett); $^{+}$ $P < 0.05$ (procedure of Nemenyi/Dunnett)

Treatment with glufosinate-ammonium did not affect the levels of ammonium in liver, kidneys or brain.

The glutamate levels in brain were reduced in the mid-dose (11–18%) and high-dose groups (20–26%). Brain levels were lower throughout the test period. An increase in the liver glutamate level was observed in the high-dose group, especially on the 1st day after treatment. On days 1 and 3, kidney glutamate levels were slightly increased (up to 21%) at 800 and 1600 mg/kg bw. Brain acetylcholinesterase levels were not affected by glufosinate-ammonium treatment.

No NOAEL could be identified. The LOAEL was 200 mg/kg bw, the lowest dose tested, based on clinical signs (enhanced spontaneous activity and isolated piloerection on day 1 after treatment) and a greater than 50% reduction in glutamine synthetase activity in liver (Ebert et al., 1986a).

Groups of 40 male and 40 female Wistar rats were administered glufosinate-ammonium (batch Lfd 13143; purity 96.9%) via the diet over 28 days at a concentration of 0, 40, 200, 1000 or 5000 ppm (equal to 0, 3.7, 19, 93 and 443 mg/kg bw per day for males and 0, 3.6, 18, 89 and 424 mg/kg bw per day for females, respectively). At the end of 28 days, 10 male and 10 female rats per

dose group were allocated to each of four subgroups corresponding to recovery periods of 0, 3, 7 or 28 days. Animals were observed daily for mortality/viability and clinical signs. Detailed clinical examinations were performed weekly, including a comprehensive observational assessment for neurological disturbance. Males and females in the 0, 1000 and 5000 ppm groups were subjected to neurobehavioural assessments weekly through the treatment period and during the first 2 weeks of recovery. Body weight and feed consumption were recorded twice weekly, and water consumption was recorded once weekly. At the end of each recovery period, all animals were killed, and livers, kidneys and brain (left and right hemispheres) were removed for examination and weighing. The catecholamine levels in the brain (five rats of each sex per group), glutamine synthetase activity (five rats of each sex per group), ammonium levels (five rats of each sex per group) and amino acid levels (five rats of each sex per group) in brain, liver and kidneys, and enzyme activity in the liver were investigated. Statements of adherence to QA and GLP were included.

During the 1st week of treatment, there was a temporary reduction in feed consumption in both sexes and a slight retardation of body weight gain in male rats in the highest-dose group. The highest-dose males showed a slight increase in water consumption throughout the whole treatment period. The comprehensive observational assessments revealed faint signs of central nervous system excitation together with reduced body temperatures (males) up to day 18 of treatment in the animals treated at 5000 ppm, but these subsided rapidly afterwards. Macroscopic examination revealed no abnormalities. A marginal increase in kidney weights at the end of treatment in the females of the highest-dose group was noted. Examination of the catecholamine transmitters revealed only a slight lowering of dopamine in females in the highest-dose group, which was no longer apparent after 3 days of recovery. There were no changes in the cerebral levels of glutamate or any other amino acids with excitatory or inhibitory neurotransmitter function. A statistically significant dose-related inhibition of glutamine synthetase activity was found in the liver (both sexes) and kidneys (males only) from 200 ppm onwards and in the brain at 5000 ppm (males only). In all cases, definite signs of reversibility were seen 7 days after termination of treatment, and there was no effect detectable at the end of the 4-week recovery period (Table 20). The inhibition of glutamine synthetase led to glutamine depletion in the affected organs, but this effect was no longer in evidence following a 3-day recovery period (Table 21).

No indications of increased glutamate or ammonium levels, a possible hypothetical consequence of glutamine inhibition, were found in any organ. Enzyme biochemistry in the liver indicated no effects on any parameters in connection with either energy and carbohydrate metabolism or glutathione metabolism (e.g. glutathione depletion). Glufosinate-ammonium did not affect alanine aminotransferase (ALT), AST or glutamate dehydrogenase or the levels of free glutamate, asparagine, aspartate or alanine in liver samples. The effects were largely reversible after 7 days of recovery and fully reversed to normal values after 28 days.

The NOAEL was 200 ppm (equal to 18 mg/kg bw per day), based on a greater than 50% inhibition of glutamine synthetase in the liver at 1000 ppm (equal to 89 mg/kg bw per day) (Ebert et al., 1986b).

Dogs

In an acute toxicity test, a single oral dose of glufosinate-ammonium (purity 95.3%) was administered by gavage to Beagle dogs (two of each sex per dose level) at 400 or 350 mg/kg bw (10% solution in a 4% carboxymethylcellulose vehicle). In addition to the standard examinations for such an acute toxicity study, a neurological examination was performed. Statements of adherence to QA and GLP were included.

All dogs, except one male, died. Glufosinate-ammonium caused clonic and tonic convulsions followed by post-epileptic inactivity. The convulsions had a delayed onset, with the maximum toxicity appearing approximately 24 hours following treatment. Short periods of stereotypic behaviour were also noted. Postural and phasic reflexes were attenuated rather than enhanced. Marked miosis preceded death in four of eight dogs. Anisocoria was observed for one female treated with 350 mg/kg bw. Death probably resulted from cardiovascular depression. Death from respiratory failure cannot be excluded (Sachsse, 1986b).

Table 20. Effect of glufosinate-ammonium on glutamine synthetase activity in the rat

Organ	Recovery (days)	Sex	Glutamine synthetase activity (mean ± standard deviation)[a]				
			0 ppm	40 ppm	200 ppm	1000 ppm	5000 ppm
Liver	0	Male	5.9 (± 0.47)	5.2 (± 0.36)	4.1*** (± 0.78)	2.9*** (± 0.31)	2.9*** (± 0.33)
		Female	5.2 (± 0.52)	5.4 (± 0.34)	3.2** (± 0.31)	2.1** (± 0.39)	1.8** (± 0.36)
	3	Male	5.9 (± 0.26)	5.7 (± 0.23)	4.2* (± 0.26)	3.4* (± 0.41)	2.6* (± 0.21)
		Female	5.8 (± 0.26)	5.3 (± 0.26)	3.3* (± 0.23)	2.3* (± 0.26)	2.6* (± 0.26)
	7	Male	5.7 (± 0.7)	5.7 (± 0.26)	4.7* (± 0.23)	4.4* (± 0.21)	4.1* (± 0.47)
		Female	5.4 (± 0.52)	6.0 (± 4.6)	4.6* (± 0.18)	4.0* (± 0.67)	3.8* (± 0.49)
	28	Male	6.1 (± 0.11)	—	5.3 (± 0.39)	5.3 (± 0.34)	5.9 (± 0.52)
		Female	5.7 (± 0.16)	—	5.7 (± 0.41)	5.5 (± 0.36)	5.3 (± 0.41)
Kidney	0	Male	3.9 (± 0.47)	3.6 (± 0.34)	3.5 (± 0.62)	3.1 (± 0.46)	3.0* (± 0.31)
		Female	1.6 (± 0.18)	1.8 (± 0.26)	1.8 (± 0.26)	1.9 (± 0.31)	1.8 (± 0.26)
	3	Male	4.1 (± 0.26)	4.0 (± 0.21)	3.1* (± 0.23)	3.0* (± 0.23)	2.6* (± 0.21)
	7	Male	5.2 (± 0.37)	3.8* (± 0.26)	4.1* (± 0.39)	3.4* (± 0.21)	3.3* (± 0.21)
	28	Male	4.6 (± 0.44)	4.2 (± 0.26)	4.1 (± 0.34)	4.0 (± 0.34)	4.4 (± 0.41)
Brain	0	Male	3.6 (± 0.52)	3.9 (± 0.37)	3.6 (± 0.31)	3.4 (± 0.44)	2.1* (± 0.34)
		Female	3.4 (± 0.34)	3.9 (± 0.41)	3.1 (± 0.31)	3.6 (± 0.6)	3.1 (± 0.49)
	3	Male	3.5 (± 0.26)	3.4 (± 0.16)	3.4 (± 0.16)	3.3 (± 0.18)	2.6* (± 0.26)
	7	Male	4.1 (± 0.49)	—	—	—	3.6 (± 0.48)
	28	Male	3.4 (± 0.16)	—	—	—	3.7 (± 0.39)

From Ebert et al. (1986b)
* $P < 0.05$ (according to Dunnett); ** $P < 0.05$ (according to Nemenyi/Dunnett); *** $P < 0.05$ (according to Sidak)
[a] Expressed as milligrams of γ-glutamylhydroxamate per gram tissue.

Table 21. Effect of glufosinate-ammonium on glutamine level in males

Organ	Recovery (days)	Glutamine level (mean ± standard deviation) (mg/g amino acid)				
		0 ppm	40 ppm	200 ppm	1000 ppm	5000 ppm
Liver	0 c, d	2.778 (± 0.497)	2.460 (± 0.561)	2.090* (± 0.355)	1.825* (± 0.225)	1.082* (± 0.320)
	3 a, d	2.116 (± 0.592)	2.404 (± 0.535)	2.276 (± 0.178)	2.092 (± 0.184)	2.052 (± 0.419)
Kidney	0 c, d	0.714 (± 0.357)	0.594 (± 0.413)	0.624 (± 0.392)	0.550 (± 0.285)	0.448* (± 0.156)
	3 b	0.328 (± 0.052)	0.322 (± 0.033)	0.232 (± 0.063)	0.252 (± 0.044)	0.350 (± 0.044)
Brain	0 e	2.794 (± 1.282)	2.464 (± 1.174)	1.720 (± 0.955)	1.364 (± 0.555)	1.264* (± 0.592)
	3 d	2.958 (± 0.629)	2.956 (± 0.63)	2.606 (± 0.742)	2.444 (± 0.728)	2.432 (± 0.636)

From Ebert et al. (1986b)
* $P < 0.05$; a: significant according to the parametric procedure of Dunnett; b: significant according to the distribution-free procedure of Nemenyi/Dunnett; c: significant according to the parametric procedure of Sidak; d: significant according to the parametric procedure of Dunnett (one-sided for decrease); e: significant according to the distribution-free procedure of Nemenyi/Dunnett (one-sided for decrease)

(b) Mechanistic studies

Mice

In a published study, the role of glutamine synthetase (GS) in early mouse embryogenesis was examined, because glutamine synthetase is expressed in a tissue-specific and developmentally controlled manner, functions to remove ammonia and glutamate and is the only enzyme that can synthesize glutamine, and because congenital deficiency of glutamine synthetase has not been reported. Because glutamine synthetase is expressed in embryonic stem cells, a null mutant mouse was generated by replacing one glutamine synthetase allele in-frame with a β-galactosidase-neomycin fusion gene. $GS^{+/LacZ}$ mice were phenotypically normal and fertile, but $GS^{LacZ/LacZ}$ mice died at embryonic day (ED) 3.5, demonstrating that glutamine synthetase is essential in early embryogenesis. Cells from ED 2.5 $GS^{LacZ/LacZ}$ embryos survive in vitro in glutamine-containing medium. Chimeric embryos constructed by injecting wild-type +/+ blastocysts with +/− or −/− cells were viable, although the glutamine synthetase–deficient cells showed a reduced fitness in chimera analysis. This indicates that the wild-type cells in the blastocyst were partly able to compensate for glutamine synthetase–deficient cells. In contrast, maternal glutamine synthetase activity could not compensate for glutamine synthetase–deficient cells. The survival of heavily (> 90%) chimeric mouse embryos up to at least ED 16.5 indicated that, after implantation, glutamine synthetase activity is not essential until at least the fetal period. The study authors hypothesized that glutamine synthetase–deficient embryos die when they move from the uterine tube to the harsher uterine environment, where the embryo has to catabolize amino acids to generate energy and, hence, needs to detoxify ammonia, two processes that require glutamine synthetase activity (He et al., 2007).

In vitro exposure of 8-day-old mouse embryos, micromass cultures in midbrain and limb bud cells to glufosinate-ammonium for 48 hours caused significant overall embryonic growth retardation and increased embryolethality to 37.5% at 10 µg/ml (5.0×10^{-5} mol/l). All embryos in the treated groups exhibited specific morphological defects, including hypoplasia of the prosencephalon (forebrain) (100%) and visceral arches (100%). In 10-day-old embryos cultured for 24 hours, glufosinate reduced the crown–rump length and the number of somite pairs and produced a high incidence of morphological defects (84.6%) at 10 µg/ml. In micromass culture, glufosinate-ammonium inhibited the differentiation of midbrain cells in day 12 embryos (Watanabe & Iwase, 1996).

In vitro exposure of mouse embryos to glufosinate-ammonium induced chromatin condensation and segregation, extracellular apoptotic bodies and cell fragments phagocytosed in macrophages in the neuroepithelium of the brain vesicle and neural tube (Watanabe, 1997).

Rats

In a study from the public literature, 7-day-old female Wistar-Immamichi rats (6–11 per group) received subcutaneous injections of glufosinate-ammonium at 0, 1, 2 or 5 mg/kg bw per day for 7 days. At 5 or 6 weeks of age, all the treatment groups showed a non-dose-dependent decreased frequency of kainic acid–induced wet-dog shakes. At 5 mg/kg bw per day, a reduction in body weight was found. The study suggests that glufosinate-ammonium treatment during the infantile period in the rat induces alterations in the kainin receptor in the brain (Fujii, Ohata & Horinaka, 1996).

In a study from the public literature, glufosinate-ammonium (30–3000 nmol/10 µl, purity unknown) was administered in vivo in the rat (Fischer 344) cerebellum through the microdialysis probe at a rate of 1 µl/minute for 10 minutes. Glufosinate-ammonium stimulated nitric oxide production, which was suppressed by an inhibitor of nitric oxide synthase and was antagonized by *N*-methyl D-aspartate (NMDA) receptor antagonists. These results suggest that glufosinate-ammonium stimulates nitric oxide production through NMDA receptors (Nakaki et al., 2000).

The effect of intravenous or intracerebroventricular injection of glufosinate-ammonium on catecholamine levels and glutamine synthetase activity in the brain was investigated in male Wistar rats.

In a preliminary study, groups of two animals each received intracerebroventricular injections of 10 or 20 µg of either glufosinate-ammonium (batch Lfd 13143; purity 96.9%) or MPP (batch Lfd 12956; purity > 99%) and were kept under observation for up to 24 hours.

After this preliminary study, groups of six animals received intracerebroventricular injections of 10 or 20 µg glufosinate-ammonium, 20 µg MPP or 10 µl physiological saline. The animals were killed 3 hours after treatment and the brains removed for determination of catecholamine (noradrenaline, dopamine and dihydroxyphenyl acetic acid) levels and glutamine synthetase activity. A parallel study was conducted with intravenous injections of glufosinate-ammonium at 0, 10 and 100 mg/kg bw in three groups of five rats each. The animals were killed 2 hours after injection, brains were dissected and catecholamine levels and glutamine synthetase activity were measured.

In the preliminary study, intracerebroventricular injection of 20 µg of glufosinate-ammonium resulted 3.5–4 hours later in sustained (> 24 hours) convulsions, which were to some extent successfully antagonized by diazepam (10 mg/kg bw intraperitoneally). From 10 minutes to 2 hours after intracerebroventricular injection of 10 µg glufosinate-ammonium, clonic spasms of forelimbs and opisthotonus were noted in one out of the two treated animals; these clinical signs disappeared rapidly after injection of diazepam. After intracerebroventricular injection of 20 µg MPP, relatively slight spasms of the forelimbs and opisthotonus were briefly observed 5 and 30 minutes after injection, respectively, in only one out of two animals. No clinical signs were observed 1 or 24 hours after injection or in the other animal at the same dose level.

In the main study, catecholamine determination on various cerebral regions—frontal cortex, striatum and hippocampus—revealed a 63% higher dihydroxyphenyl acetic acid concentration in the striatum and a 31% lower noradrenaline concentration on the frontal cortex 3 hours after intracerebroventricular injection of 20 µg of glufosinate-ammonium. Following 10 µg of glufosinate-ammonium and 20 µg of MPP, there were no changes in the catecholamine concentrations in any of the cerebral regions examined. There were also no changes in the catecholamine concentrations in any of these cerebral regions 2 hours after intravenous injection of glufosinate-ammonium at 10 or 100 mg/kg bw.

After intravenous injection, only one out of five animals from the highest dose tested (100 mg/kg bw) showed a decreased glutamine synthetase activity. When administered by intracerebroventricular injection, glufosinate-ammonium induced a dose-related inhibition of glutamine synthetase activity. Intracerebroventricular injection of MPP at the same high dose as glufosinate-ammonium (20 µg/animal) did not produce any inhibition of glutamine synthetase activity, and no clinical signs of intoxication were observed in any of the animals treated with MPP (Gerhards, Koecher & Ulm, 1986).

In a supplemental study, the sensitivity of the Fischer rat strain with regard to the inhibition of glutamine synthetase activity in the liver was investigated. Groups of 10 male and 10 female Fischer 344 rats were administered glufosinate-ammonium (purity 92.1%) via the diet over 90 days at a concentration of 0, 8, 64, 500 or 4000 ppm. Five rats of each sex per group were killed at the end of the 13-week study period, and five animals of each sex per group were killed after an additional 4-week post-dosing recovery period. At termination, serum and liver samples were obtained for measurement of liver glutamine synthetase activity and serum ammonia concentration.

Glutamine synthetase activity was statistically significantly inhibited at the end of the 13-week treatment period in the liver of both sexes from the 4000 ppm group and from the 500 ppm females. After the 4-week recovery phase, no inhibition was noted. Serum ammonia levels were not affected by treatment (Ohashi, Nakayoshi & Abe, 1982).

Glufosinate-ammonium (purity 96.9%) was tested in various in vitro receptor binding assays for γ-aminobutyric acid, noradrenaline (NA-α2 and NA-β), dopamine and serotonin (5-HT1 and 5-HT2). The affinity of glufosinate-ammonium for the benzodiazepine-binding site and the calcium ion channel was also investigated.

Glufosinate-ammonium (1 µmol/l) caused no displacement of the ^{3}H ligands from the receptor in any of the investigated receptor systems (Schacht, 1986).

The effect of glufosinate-ammonium (purity 99.5%) on the substrates of the Krebs cycle in isolated rat liver mitochondria and the oxidative phosphorylation of intact mitochondria was investigated.

No indication that glufosinate-ammonium affects oxidative metabolism in mitochondria was found. It was concluded that glufosinate-ammonium had no direct influence on the metabolism of intact rat liver mitochondria, or glufosinate-ammonium had not been taken up by these organelles (Metzger, 1986).

In a study in vitro, glufosinate-ammonium (10 mmol/l) did not inhibit the activity of ALT, AST, glutamate dehydrogenase or γ-glutamyl transpeptidase (Koecher, 1986).

In vitro, phosphinothricin, the free acid form of glufosinate-ammonium, was found to be a strong inhibitor of mammalian glutamate decarboxylase, which converts glutamate to γ-aminobutyric acid (Lacoste et al., 1985).

In in vitro tests, phosphinothricin, the free acid form of glufosinate-ammonium, as well as other analogues of glutamic acid were shown to be effective inhibitors of glutamine synthetase, acting competitively against L-glutamate. No effect was observed on the activity of glutamate dehydrogenase, whereas inhibition of decarboxylase and transaminases was insignificant (Lejczak, Starzemska & Mastalerz, 1981).

The effect of glufosinate-ammonium (concentration 50.2% w/w) at doses up to 500 µg/ml on the in vitro activity of glutamine synthetase was investigated in samples of liver, kidney and brain (neocortex, medulla oblongata and hypothalamic region) of Wistar rats. Statements of adherence to QA and GLP were included.

Glufosinate-ammonium induced a significant dose-related inhibition of glutamine synthetase from 10 µg/ml onwards. The maximal level of inhibition was 72%. Glufosinate-ammonium induced no inhibition of kidney glutamine synthetase activity at 1, 3 or 10 µg/ml. A dose-related inhibition was observed from 100 µg/ml onwards, with a maximum of 17%.

In the brain (neocortex, medulla and hypothalamus), glufosinate-ammonium induced a dose-related inhibition from 30 µg/ml onwards The maximal level of inhibition was in the range of 41–53% (Luetkemeier, 1999).

The kinetics of the decrease in serum glutamine levels after a single oral dose of glufosinate-ammonium (purity 95.2%) was investigated. Groups of four non-pregnant female Wistar rats received a single oral (gavage) dose of 200 mg/kg bw of glufosinate-ammonium or vehicle (0.5% methylcellulose). All animals were observed for mortality and clinical signs. Body weights were recorded before the study start, on the day of treatment (study day 1) and on study day 2. Blood

samples were collected from all animals in each group prior to treatment and then 1, 2, 4, 8 and 24 hours after treatment. Glutamine levels in the serum were determined by HPLC.

No mortalities and no clinical signs were observed. A progressive decrease in the mean serum glutamine levels was observed in the group treated with glufosinate-ammonium compared with the control group. The lower glutamine level was reached between 4 and 8 hours after treatment and remained stable until the 24-hour time point. When compared with the control group, a 24–31% decrease in glutamine level was observed between 4 and 24 hours after a single oral dose of glufosinate-ammonium (Kennel, 2003a).

The kinetics of the decrease in serum glutamine levels after a single oral dose of glufosinate-ammonium was investigated. Groups of 18 pregnant Wistar rats received a single oral (gavage) dose of 200 mg/kg bw of glufosinate-ammonium (batch 2/93; purity 95.2%) or vehicle (0.5% methylcellulose) on GD 6. All animals were observed for mortality and clinical signs. Body weights were recorded before the study start, on the day of treatment (study day 1) and on study day 2. Blood samples were collected from all animals 24 hours after treatment. Glutamine levels in the serum were determined by HPLC. Statements of adherence to QA and GLP were included.

No mortalities and no clinical signs occurred during the study. The mean serum glutamine level in the group treated with glufosinate-ammonium was significantly ($P < 0.01$) reduced by 21% compared with the control group (Kennel, 2003b).

(c) The significance of glutamine synthetase inhibition to humans

A number of studies concerning the significance of glutamine synthetase activity in humans were available. Based on the available information, JMPR in 1999 came to the following conclusion:

> Glutamine synthetase is a key enzyme in the metabolism of nitrogen and glutamate, catalysing the multi-step reaction of
>
> (−)-glutamate + ATP + NH_3 ↔ (−)-glutamine + ADP + P
>
> In plants, glutamine synthetase is the main enzyme involved in the control of ammonia concentrations, and its inhibition is the mechanism of action of glufosinate-ammonium in plants. In mammals, other pathways exist for the homeostatic control of ammonia, such as reverse reaction of amino acid dehydrogenases and the carbamoyl phosphate synthetase–urea cycle. Glutamate and glutamine can, however, play significant roles in other biochemical and physiological processes in mammals, such as neurotransmission (glutamate and gamma-aminobutyric acid (GABA)). The activity of glutamine synthetase varies between tissues and species (see below), but the amino acid sequence is reported to be well conserved (Lie-Venema et al., 1998; Purich, 1998; Ernst & Leist, 1999a).
>
> The liver has two distinct systems for dealing with ammonia. A high-capacity, low-affinity system exists in the periportal hepatocytes which is based on carbamoyl phosphate synthetase and the urea cycle. In central vein hepatocytes, a low-capacity, high-affinity system exists which is based on glutamine synthetase and ornithine aminotransferase. [Hack, Ebert & Ehling] (1994) showed that doses of glufosinate-ammonium did not increase ammonia concentrations in liver at a dose (5000 ppm) that inhibited glutamine synthetase activity by 50%. While a 60% reduction in liver glutamine was seen at day 1, the concentration had returned to normal by day 4, indicating the induction of alternative pathways. Inhibition of liver glutamine synthetase by up to 50% is therefore not considered to be adverse in isolation.
>
> The activity of this enzyme in kidney varies considerably between species (Lie-Venema et al., 1998; see below), with relatively high activity in rodents but negligible activity in dogs and humans. Inhibition of kidney glutamine synthetase in the absence of pathological findings is not considered to be relevant to human risk assessment.
>
> In the brain and central nervous system, ammonia homeostasis is controlled by a number of enzymes including glutamine synthetase and glutamate dehydrogenase. Under normal conditions (~ 100 µmol/L of

ammonium and 3 mmol/L of glutamate), the flux through glutamine synthetase in brain is 2–10% of its theoretical capacity and that of glutamate dehydrogenase is approximately 0.1% of its capacity (Lie-Venema et al., 1998). With such excess capacity, inhibition of brain glutamine synthetase is unlikely to result in significant increases in brain ammonia concentrations. This conclusion is supported by the finding of [Hack, Ebert & Ehling] (1994) that brain ammonia concentrations were not increased at doses of glufosinate-ammonium that produced a 40% reduction in brain glutamine synthetase activity in rats. However, the glutamine–glutamate shunt between GABA and glutamate in neurons and glutamine in astrocytes plays a role in both excitatory and inhibitory neurotransmission. The results of [Hack, Ebert & Ehling] (1994), although somewhat inconsistent, indicate that significant changes in a range of biogenic amines in regions of the dog brain are associated with changes of ≥ 8% in glutamine synthetase activity after administration of glufosinate-ammonium at 8 mg/kg bw for 28 days, a dose that produced "increased gait activity". It is thus proposed that any statistically significant, > 10% inhibition of glutamine synthetase activity in brain is a marker of potentially adverse effects on brain biochemistry and behaviour.

3. Observations in humans

Medical examinations of plant production personnel have not found effects related to the production of glufosinate-ammonium.

Several human poisoning cases due to (suicidal) ingestions of glufosinate-ammonium have been described in the literature. Observed symptoms were nausea, vomiting, diarrhoea, abdominal pain, tremor, hypotonia, bradycardia or tachycardia, muscle weakness, drowsiness, loss of consciousness and coma, convulsions and respiratory arrest. Rarely, hyperthermia has been described. The neurological symptoms may suddenly appear with a delay (latency period) of 8–48 hours without prior warning. Both cytotoxic and vasogenic oedema have been reported in brain regions. The fatality rate in reported poisonings is about 18%. It is not clear whether the toxicity is due to the active ingredient, to the surfactant contained in relatively high amounts in the formulation or to the combination of both (Koyama et al., 1994).

B. *N*-ACETYL-GLUFOSINATE (NAG)

N-Acetyl-L-glufosinate (NAG) is the major metabolite in genetically modified crops sprayed with racemic glufosinate-ammonium. Following oral administration of glufosinate-ammonium to rats, NAG is observed in the urine at extremely low trace levels. It was not detected in any organ extracted. Therefore, NAG is considered as a non-common metabolite. It cannot be assessed as a bioavailable metabolite in studies with parent glufosinate-ammonium and is consequently not covered in toxicity studies with glufosinate-ammonium in rats. Therefore, a toxicology dossier as would typically be required for non-common metabolites was submitted. It is noted that NAG is usually supplied as an aqueous solution. The dose levels referred to in the studies refer to the active ingredient unless stated otherwise.

The structure of ^{14}C-labelled NAG is shown in Figure 3.

Figure 3. Chemical structure of [^{14}C]N-acetyl-glufosinate (NAG). The positions of the radiolabel ^{14}C (C*) used in the toxicokinetics studies are shown.

H_3C P O O$^-$ Na$^+$ * * O O$^-$ Na$^+$ HN O CH_3

4. Biochemical aspects

4.1 Absorption, distribution and excretion

Rats

Groups of male and female Wistar rats were administered a single oral or intravenous dose of [3,4-^{14}C]NAG (purity ~98%) at 3 mg/kg bw. The substance was dissolved in a pyrogen-free saline. The positions of the ^{14}C labels are shown in Figure 3. An overview of the study groups is presented in Table 22. In four groups of five males or five females, excretion was studied over a period of 4 days. At the end of the study, animals were sacrificed and dissected to sample different organs and tissues for determination of the distribution of the radioactive residues. Whole-body autoradiography was performed in another two males 4 days after oral and intravenous administration, respectively. Metabolism was investigated by radio-HPLC of the contents of stomach and intestine in two additional male rats dosed orally and sacrificed, respectively, 4 hours and 24 hours after dosing.

Table 22. Overview of experimental groups

Test group	Number and sex	Administration route	Targeted dose (actual applied doses)	Sampling regimen
1	5M/5F	Oral gavage	3 mg/kg bw (single), [^{14}C]NAG (2.5–3.0 mg/kg bw)	Excretion and residues Urine, cage washings and faeces collected at 4 and 8 h and 1, 2, 3 and 4 days after dosing. Sacrifice and tissue and blood collection on day 4.
	2M	Oral gavage	3 mg/kg bw (single), [^{14}C]NAG (3.0 mg/kg bw)	Metabolism One rat was killed 4 h after dosing and one 24 h after dosing. Gastrointestinal contents were removed and investigated for metabolism.
	1M	Oral gavage	3 mg/kg bw (single), [^{14}C]NAG (2.8 mg/kg bw)	Whole-body autoradiography 96 h after dosing
2	5M/5F	Intravenous	3 mg/kg bw (single), [^{14}C]NAG (2.8–3.3 mg/kg bw)	Excretion and residues Urine, cage washings and faeces collected at 4 and 8 h and 1, 2, 3 and 4 days after dosing. Sacrifice and tissue and blood collection on day 4.
	1M	Intravenous	3 mg/kg bw (single), [^{14}C]NAG (3.1 mg/kg bw)	Whole-body autoradiography 96 h after dosing

From Kellner, Stumpf & Braun (1993)
F, female; M, male

Organs/tissues (blood, bone, brain, carcass, fat, gonads, heart, kidney, liver, lung, plasma, skeletal muscle, spleen) of each rat were collected. Radioactivity levels were determined by LSC. Metabolite characterization was performed in stomach and intestines. NAG dosing solutions were homogeneous, and radiolabelled solutions remained stable during the study. Statements of adherence to QA and GLP were included.

No abnormalities were observed in the rats upon oral or intravenous exposure. The kinetics results are summarized in Table 23. Absorption by orally treated rats was slight, with mean values of 5.5% for males and 6.3% for females. Over a period of 96 hours, orally administered radioactivity was excreted mainly via faeces, with values of 98% ± 5% in males and 109% ± 11% in females. The mean renal portion was about 6% in both males and females. Following intravenous administration,

radioactivity was mainly excreted via urine, with 97% ± 5% in males and 95% ± 7% in females. The mean faecal portion was between 1.8% and 4.1%.

Table 23. Half-lives for excretion in urine and faeces after oral and intravenous administration of [^{14}C]NAG

	Half-life (mean ± standard deviation) (h)			
	Oral administration, single dose (3 mg/kg bw)		Intravenous administration, single dose (3 mg/kg bw)	
	Males	Females	Males	Females
Phase I, urine	3.6 ± 0.7	3.8 ± 0.3[a]	1.7 ± 0.6	1.4 ± 0.4
Phase II, urine	18.0 ± 3.5	25.6 ± 9.3[a]	18.2 ± 2.0	42.3 ± 39.2[b] 24.8 ± 3.9[b]
Phase I, faeces	5.1 ± 0.3	6.2 ± 0.8[a]	12.1 ± 2.4	12.6 ± 1.5

From Kellner, Stumpf & Braun (1993)

[a] One female not included, as it showed an inverse excretory pattern (112.1 hours for phase II).

[b] Values with and without one female showing very high phase II half-life of 112.1 hours (others range from 19.8 to 28.5 hours).

In the orally dosed rats (test group 1), total recovery in urine, cage washings and faeces ranged from 100% to 125% at 96 hours, of which 94–119% (one rat [no. 13] only 20%) was found in the faeces and 5.2–6.7% in the urine (one rat [no. 13] 85%). In the intravenously dosed rats (test group 2), total recovery in urine, cage washings and faeces ranged from 94% to 105% at 96 hours, of which 88–104% was found in the urine and 1.0–7.3% in the faeces. Mean values per sex are presented in Table 24.

The mean total radioactivity in tissues and blood of rats dosed orally was 0.097% (excluding rat no. 13, for which it was 0.14% of the total administered radioactivity) on day 4. The mean total radioactivity in tissues and blood of rats dosed intravenously was 0.22% on day 4. Measurable concentrations of administered radioactivity were found only in kidneys, testes, liver, spleen, bones and carcasses of the males and in kidneys, liver, spleen and carcasses, but not gonads, of the females. In the other organs, no detectable concentrations were observed. One orally dosed female rat differed from all the other animals. This was probably caused by faulty intubation, as suggested by the relatively high concentration of radioactivity in the lungs. However, no abnormalities were observed during the study or at necropsy.

Whole-body autoradiography showed radioactivity in the kidneys (concentrated in the medulla), spleen (accumulated in the red pulp), salivary glands and thymus (homogeneous distribution), testes (band-like) and epididymides (circular). It was not possible to assign the radioactivity to any particular tissue structure. This also applies to the occasionally observed, but extensive, accumulations in the liver.

Except for gonads and kidneys, where concentrations were slightly higher in males than in females, no definite sex-specific differences were found (Kellner, Stumpf & Braun, 1993).

The blood-level kinetics of NAG was investigated in male and female Wistar rats following an oral or intravenous administration of [^{14}C]NAG at a targeted common dose rate of 3 mg/kg bw. Three rats of each sex per exposure group received a single dose via either gavage or injection into a tail vein. Blood samples were taken 0.25, 0.5, 1, 2, 4, 6 and 8 hours and 1, 2, 3 and 4 days after dosing. Statements of adherence to QA and GLP were included.

Table 24. Mean percentage of administered [^{14}C]NAG after a single dose (oral and intravenous)

Time after dosing (h)	Mean % of administered radioactivity					
	Oral			Intravenous		
	Faeces	Urine	Cage wash[a]	Faeces	Urine	Cage wash
Males						
0–4	—	1.956	—	—	85.6	—
4–8	—	0.606	—	—	8.89	—
8–24[a]/0–24[b]	95.21	2.476	0.0428	1.416	1.71	0.092
24–48	2.242	0.140	0.0035	0.269	0.37	0.023
48–72	0.047	0.031	—	0.057	0.13	—
72–96[a]/48–96[b]	0.0072	0.012	—	0.025	0.06	0.008
Total	97.51	5.222	0.0463	1.768	96.76	0.123
Total excreted	102.78			98.66		
Females[c]						
0–4	—	1.933	—	—	91.24	—
4–8	—	0.831	—	—	1.95	—
8–24[a]/0–24[b]	102.6	2.825	0.0746	3.44	0.79	0.122
24–48	4.37	0.219	0.0092	0.49	0.37	0.086
48–72	2.01	0.053	—	0.14	0.28	—
72–96[a]/48–96[b]	0.041	0.026	—	0.06	0.15	0.083
Total	109.1	5.887	0.0838	4.14	94.78	0.27
Total excreted	115.02			99.23		

From Kellner, Stumpf & Braun (1993).
[a] Urine and cage wash.
[b] Faeces.
[c] Means do not include the findings of rat no. 13.

Following oral administration (actual rates vary from 2.5 to 3.6 mg/kg bw), the maximum blood levels were reached after approximately 1 hour, amounting to mean concentrations of 0.052 and 0.051 mg Eq/kg in male and female rats, respectively. The last measurable concentrations were found 8 hours after dosing. The elimination kinetics was biphasic, with half-lives of 0.8 and 0.9 hour (phase I) and 6.3 and 7.4 hours (phase II) for males and females, respectively. The area under the plasma concentration–time curve ($AUC_{0-\infty}$) values amounted to 0.214 ± 0.031 mg Eq·h/kg for male rats and to 0.192 ± 0.064 mg Eq·h/kg for female rats. Following intravenous administration, the blood level at the first sampling interval, 5 minutes after injection, amounted to 6.19 and 7.44 mg Eq/kg in male and female rats, respectively, at the mean. The blood levels declined rapidly. At 8 hours post-dosing, levels of 0.008 and 0.010 mg Eq/kg were measured in male and female rats, respectively. The elimination kinetics was also biphasic, with half-lives of 0.35 and 0.30 hour (phase I) and 12.9 and 15.4 hours (phase II) for males and females, respectively. The $AUC_{0-\infty}$ values amounted to 3.66 ± 0.158 mg Eq·h/kg for male rats and 3.86 ± 0.207 mg Eq·h/kg for female rats. The kinetics results are summarized in Table 25.

The bioavailable portion after oral administration (oral absorption) took place rapidly after oral administration, but was only slight, calculated from the $AUC_{0-\infty}$ values after oral and intravenous administration. This calculation yielded a bioavailable portion of orally administered NAG of 5.9% of the dose for male rats and 5.0% of the dose for female rats (Kellner & Braun, 1993).

Table 25. Toxicokinetic data in blood after a single oral or intravenous administration of [^{14}C]NAG

Parameter	Oral administration, single dose (3 mg/kg bw)		Intravenous administration, single dose (3 mg/kg bw)	
	Males	Females	Males	Females
C_{max} (mg Eq/kg)	0.052	0.051	6.19	7.44
T_{max} (h)	1	1.17	0.083	0.083
Phase I $t_{½}$ (h)	0.82	0.86	0.35	0.3
Phase II $t_{½}$ (h)	6.3	7.4	12.9	15.4
$AUC_{0-\infty}$ (mg Eq·h/kg)	0.21	0.19	3.66	3.86
Bioavailability (%)[a]	5.9	5.0	—	—

From Kellner & Braun (1993)
$AUC_{0-\infty}$, area under the plasma concentration–time curve from time zero to infinity; C_{max}, maximum plasma concentration; Eq, equivalents; $t_{½}$, half-life; T_{max}, time to reach C_{max}
[a] Bioavailability (%) = 100 × (AUC (oral) / AUC (intravenous)).

In a parallel study, groups of male and female Wistar rats were treated with a single oral gavage dose of [3,4-^{14}C]NAG (purity > 90%) at 3 mg/kg bw. The study was aimed at determining the rate and extent of excretion of radioactivity, the metabolite profile in excreta and the identity of the major excreted metabolites. The results of the metabolite profile and the identity of the major excreted metabolites are presented in the next section. The substance was stocked as an aqueous solution, which was diluted with sodium chloride solution before administration. The positions of the ^{14}C labels are shown in Figure 3 above. Five male and five female animals were treated. Excreta were collected up to 96 hours after dosing. Native urine was investigated by radio-HPLC using an anion exchange system or by radio-TLC using silica plates. Radioactivity in cage wash and carcass was not measured, because a parallel toxicokinetic study (Kellner & Braun, 1993) had shown that radioactivity in these fractions was less than 0.5% of the dose. Statements of adherence to QA and GLP were included.

Total renal excretion accounted for 5.5% and 9.3% of the administered dose for males and females, respectively. The majority of the radioactivity was eliminated in the 0- to 24-hour interval. Total faecal excretion accounted for 100% and 95.8% of the administered dose in males and females, respectively, with the majority being excreted in the 0- to 24-hour interval. Total recoveries of the radioactivity were 105.4% and 105.1% for males and females, respectively (Stumpf, 1993b).

Pharmacokinetics in rats following a single oral administration of NAG at a high dose level was investigated. ^{14}C-labelled NAG was dissolved in saline and orally administered to different groups of male and female rats by gavage at a target dose rate of 1000 mg/kg bw (actual dose levels ranged from 1017 to 1196 mg/kg bw). Different animals of both sexes were sacrificed 2 hours (one animal of each sex), 6 hours (one animal of each sex), 24 hours (five animals of each sex) and 96 hours (five animals of each sex) after dosing. Urine, faeces and cage wash were collected on a daily basis or until sacrifice for a maximum period of 4 days. After humane killing, the animals were dissected, and selected organs and tissues were sampled. All samples were radioassayed by LSC. Statements of adherence to QA and GLP were included.

The material balance was effectively complete and amounted to 95.3% of the dose for both sexes 96 hours after dosing (Table 26). The excretion was rapid, as approximately 71% and 66% of the dose were excreted during the 1st day after dosing by male and female rats, respectively. The faeces proved to be the predominant excretion route, accounting for approximately 87% and 77% of the dose 96 hours after administration to male and female rats, respectively. Total renal excretion (urine and cage wash combined) accounted for 8.5% and 18.6% of the dose in males and females, respectively. The high figure for female rats was assessed by the study director to be a result of contamination by the faeces. Considering the excretion pattern of all animal groups in this and other

studies, a lower value for oral absorption, of 8–10% of the dose, was assumed to be realistic. The overall data also indicate that the oral absorption of NAG does not depend on the dose rate.

Table 26. Material balance and excretion of radioactive residues 0–96 hours after an oral administration of [^{14}C]NAG at 1000 mg/kg bw to male and female rats

Matrix	% of administered dose[a]							
	Males				Females			
	0–24 h		0–96 h		0–24 h		0–96 h	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Urine	6.20	2.26	6.79	1.71	7.01	2.35	12.71	6.24
Faeces	62.24	5.89	86.76	9.11	56.95	12.5	76.68	14.4
Cage wash	2.18	1.16	1.71	0.65	2.24	1.00	5.89	4.30
Total excreted	70.62	3.65	95.26	7.78	66.09	13.26	95.28	4.57
Organs/tissues	0.04	0.03	< LOD	< LOD	0.01	0.003	< LOD	< LOD
Total recovery	70.66	3.67	95.26	7.78	66.27	13.34	95.28	4.57

From Maas & Braun (1995)
LOD, limit of detection; SD, standard deviation
[a] Mean and standard deviation of five rats of each sex per collection period.

The total radioactive residue detected in organs and tissues 2 hours after administration was below 0.5% of the dose and declined rapidly. The highest residue levels were consistently observed in the kidneys and liver at all sampling times. Four days after administration, radioactivity was detectable only in the kidneys of three males, whereas in females, no radioactivity was detectable in any of the organs or tissues. These results did not indicate any potential for bioaccumulation of NAG. No marked sex-specific differences were observed. The average half-lives estimated for faecal elimination were 11 hours in males and 10 hours in females. The half-life for renal excretion was 17 hours in males and 19 hours in females (Maas & Braun, 1995a).

The metabolism of a single high-dose oral administration of [^{14}C]NAG in rats was investigated. ^{14}C-labelled NAG was dissolved in saline and orally administered to different groups of male and female rats by gavage at a target dose rate of 1000 mg/kg bw (actual dose levels ranged from 999 to 1594 mg/kg bw). Different animals of both sexes were sacrificed 2 hours (one animal of each sex), 6 hours (one animal of each sex), 24 hours (five animals of each sex) and 96 hours (five animals of each sex) after dosing. Urine, faeces and cage wash were collected on a daily basis or until sacrifice for a maximum period of 4 days. After humane killing, the animals were dissected, and selected organs and tissues were sampled. Metabolite characterization of all samples is described in section 4.2 in the evaluation of the study of Lauck-Birkel (1995b). Statements of adherence to QA and GLP were included.

The total recovery (urine, faeces and cage wash) was 98.9% and 97.7% for male and female rats, respectively. The renal excretion (including cage wash) accounted for 10% of the administered dose for both sexes. The total faecal excretion accounted for 89% and 88% of the total dose for males and females, respectively. Most of the radioactivity was eliminated within 0–48 hours (90–92% of the administered dose). Residue levels in the organs and tissues were insignificant (< 0.01% of the dose already at 24 hours after administration) (Maas & Braun, 1995b).

The absorption, distribution and elimination of a single intermediate dose (30 mg/kg bw) of [3,4-^{14}C]NAG were investigated by Maas & Braun (1999c). Three groups of five male Wistar rats received an actual NAG dose of 31.6–35.1 mg/kg bw dissolved in water by stomach tube. Animals were killed 1, 6 and 24 hours after administration. Blood and plasma, kidneys, liver and brain were

sampled. These organs were radioassayed, extracted and profiled for residue composition by HPLC with co-elution of authentic reference substances. Excreta were collected for 24 hours only from the third group, radioassayed by LSC and analysed for the composition of residues by radio-HPLC. The metabolic profiling results are summarized in section 4.2 below on biotranformation (Lauck-Birkel & Strunk, 1999d). Statements of adherence to QA and GLP were included.

Twenty-four hours after the oral administration, more than 90% of the dose was excreted: approximately 2% in the urine and 88% of the dose with the faeces. One hour after oral administration, the highest residue level was observed in the kidneys, amounting to approximately 2.01 mg Eq/kg, followed by the liver, with 0.36 mg Eq/kg, as well as blood and plasma, with 0.20 and 0.33 mg Eq/kg, respectively. Similar residue levels in blood and plasma indicated no adsorption of NAG residues to the blood cells. The lowest residues were detected in the brain, amounting to 0.02 mg Eq/kg, suggesting poor penetration of the NAG residues through the blood–brain barrier. Six hours after administration, the maximum residue level was again observed in the kidneys, accounting for 1.09 mg Eq/kg. Compared with the first sampling time, the residues slightly increased in liver and brain, amounting to 0.46 and 0.03 mg Eq/kg, whereas the residues in blood and plasma dropped to 0.10 and 0.14 mg Eq/kg, respectively.

Twenty-four hours after administration, all residue levels were less than 1 mg Eq/kg in the selected organs. The highest residue level was observed in kidneys, accounting for 0.66 mg Eq/kg, followed by the liver, with 0.21 mg Eq/kg. Other residue levels were less than 0.04 mg Eq/kg (Maas & Braun, 1999c).

The absorption, distribution and elimination of a single intravenous low dose (3 mg/kg bw) of NAG in rats were investigated by Maas & Braun (1999d). Two groups of five male Wistar rats received an actual dose of [3,4-^{14}C]NAG at 3.1–3.4 mg/kg bw dissolved in saline injected into the tail vein. Animals were sacrificed 2 and 24 hours after administration. Sacrificial blood and plasma, kidneys, liver and brain were sampled. These organs were radioassayed, extracted and profiled for residue composition by HPLC with co-elution of authentic reference substances. Excreta were collected for 24 hours only from the second group, radioassayed by LSC and analysed for the composition of residues by radio-HPLC. The metabolic profiling results of tissues, organs and excreta are summarized in section 4.2 on biotranformation (Lauck-Birkel & Strunk, 1999c). Statements of adherence to QA and GLP were included.

Twenty-four hours after intravenous administration, more than 87% of the dose was excreted. Approximately 85% of the dose was excreted with the urine and 2% with the faeces, suggesting that the biliary excretion is low. Therefore, the renal excretion after oral administration of NAG approximates the oral absorption.

Two and twenty-four hours after intravenous administration, the highest residue level was observed in the excretory organs, the kidneys, followed by the liver. Even lower levels were measured in blood and plasma (Maas & Braun, 1999d).

4.2 Biotransformation

Rats

In the study of Stumpf (1993b; see study description under section 4.1), metabolites were analysed in urine and faeces of Wistar rats dosed orally with [^{14}C]NAG (purity > 90%) at a targeted rate of 3 mg/kg bw. Metabolites were isolated by HPLC and identified by co-chromatography.

After oral administration of NAG at actual dose rates of 2.1–3.4 mg/kg bw, the main metabolite was the unchanged NAG (70% of applied dose), predominantly in the (0- to 24-hour) faeces. In urine, NAG was the main component, accounting for 3.5% of the dose in males and 6.6% in females. Two impurity peaks (MPP and MPB) of the test substance were observed in urine at very low dose levels (0.6–0.7% of the dose). It is unclear whether these levels should be attributed to metabolism or to impurities present in the test substance. Free glufosinate in urine was detected at or

slightly above the limit of detection of 0.05% of the dose. Radio-HPLC and radio-TLC of the purified faeces extract showed that NAG formed the main residue, with 68.2% of the dose in males and 68.4% of the dose in females. Free glufosinate was detected in faeces at 10.8% and 9.3% of the dose, respectively, for males and females. In addition, two minor metabolites, MHB and MPP, accounted for, respectively, 0.7–1% and 0.2–0.6% of the dose (Stumpf, 1993b).

The metabolism of a single high-dose oral administration (1000 mg/kg bw) of [^{14}C]NAG in urine, faeces and cage wash samples of rats was investigated. The study design is described in section 4.1 (Maas & Braun, 1995b). All samples were radioassayed by LSC. Urine and aqueous extracts of faeces were investigated by radio-HPLC and/or radio-TLC. Statements of adherence to QA and GLP were included.

The metabolic profile in the excreta was the same in both sexes. Unchanged NAG was the predominant residue component in the 0- to 96-hour excreta, accounting for 6.7% and 7.4% of the dose in the urine and 84.9% and 88.5% of the dose in the faeces of males and females, respectively. Minor metabolites (< 0.5% of the dose) detected in urine and faeces were MHB, MPP, MPA and MPB. The low levels suggest that NAG was essentially not metabolized in the rat. No deacetylated NAG (glufosinate) was detected in urine. Deacetylation of NAG was observed in the intestine, but only up to 1% of the dose (Lauck-Birkel, 1995b).

The metabolism of a single intermediate oral dose (30 mg/kg bw) of [3,4-^{14}C]NAG was investigated in the tissues, organs and excreta collected in the study described in the previous section (Maas & Braun, 1999c). These organs were radioassayed, extracted and profiled for residue composition by HPLC with co-elution of authentic reference substances. Excreta were collected for 24 hours only from the third group, radioassayed by LSC and analysed for the composition of residues by radio-HPLC. The metabolic profile in excreta, blood and plasma, liver and kidney is described here. Statements of adherence to QA and GLP were included.

Unchanged NAG was the predominant residue component in urine (1.7% of the dose) and faeces (82% of the dose). Minor metabolites were detected in urine: MPP (0.15%), MPA (0.03%), MPB (0.18%) and L-glufosinate (0.02% of the dose). In the faeces, the deacetylated substance, L-glufosinate, amounted to 5.2% of the dose. MPB was again detected in the faeces, amounting to 1.4% of the dose, possibly resulting from the MPB impurity of the test substance. In the kidneys, the unchanged test substance NAG, representing 0.031% of the dose, was the main compound 1 hour after dosing. In addition, traces of MPP, L-glufosinate (free acid) and MPB were also detected. Six hours after dosing, the metabolite profile was comparable, whereas 24 hours after dosing, L-glufosinate (free acid) was the major residue component, amounting to 0.018% of the dose. Some traces of NAG and MPP were still observed. In the liver, NAG and MPP proved to be the two main residue components 1 hour after administration, accounting for 0.018% of the dose each. Traces of MPB and L-glufosinate (0.001% of the dose) were also detected. Six hours after administration, MPP was the main metabolite in the liver, amounting to 0.038% of the dose. Unchanged NAG accounted for 0.011% of the dose, and traces of MPB were again observed. Twenty-four hours after dosing, MHB was the main metabolite in the liver, representing 0.015% of the dose, although it was not detected at earlier sampling periods. Glufosinate was no longer detected 6 and 24 hours after administration (Lauck-Birkel & Strunk, 1999d).

The metabolism of a single low intravenous dose (3 mg/kg bw) of [3,4-^{14}C]NAG was investigated in the tissues, organs and excreta collected in the study of Maas & Braun (1999d). These samples were radioassayed, extracted and profiled for residue composition by HPLC with co-elution of authentic reference substances. Excreta were collected for 24 hours only from the second group, radioassayed by LSC and analysed for the composition of residues by radio-HPLC. The metabolic

profile in excreta, blood and plasma, liver and kidney is described here. Statements of adherence to QA and GLP were included.

The unchanged test substance NAG was the principal residue component in urine (approximately 85% of the dose) and faeces (approximately 2% of the dose). A minor metabolite detected in urine was MPB, amounting to approximately 1% of the dose. However, MPB was reported to be an impurity of the test substance (1.2%). Apart from the test substance NAG, two minor metabolites were additionally observed in the faeces: i.e. L-glufosinate (representing the free acid) and MPB, each amounting to less than 0.1% of the dose.

In kidneys and liver, the unchanged test substance NAG proved to be the main residue component, amounting to 0.79% and 0.01% of the dose, respectively, 2 hours after intravenous administration. Apart from NAG, L-glufosinate (representing the free acid) was detected as the main metabolite in the kidneys (but not in the liver), accounting for 0.05% and 0.06% of the dose 2 and 24 hours after dosing, respectively. Additionally, MPP was detected in the liver at a low level of 0.04% of the dose. With the exception of glufosinate, all residue levels had decreased significantly by the 24-hour sampling (Lauck-Birkel & Strunk, 1999c).

The results of the above studies suggest the same metabolic pathway of NAG in the rat for both oral and intravenous routes of exposure (Figure 4). NAG is partly deacetylated to form glufosinate, which is further metabolized via oxidative deamination and decarboxylation, resulting in MPP and MPA (Lauck-Birkel & Strunk, 1999c,d).

5. Toxicological studies

5.1 Acute toxicity

(a) Lethal doses

Two acute oral toxicity studies with NAG were performed in rats and mice, respectively. The results are summarized in Table 27. No treatment-related mortality occurred in either study, and no macroscopic findings were reported. Treatment-related clinical signs of toxicity observed in surviving rats were reduced spontaneous activity, high-legged gait, contracted flanks, squatting position, piloerection, irregular breathing and increased respiratory rate on day 1 only. After the day of treatment, animals were free of clinical signs. In mice, largely the same clinical signs were observed (reduced spontaneous activity, contracted flanks, squatting position, piloerection and increased respiratory rate), but some of the signs were still observed 14 days after treatment.

(b) Dermal sensitization

A dermal sensitization study (maximization test) was performed according to OECD Test Guideline No. 406. Female Pirbright-White guinea-pigs were used: 9 in the dose range–finding tests, 10 in the control group and 20 in the treatment group. An additional escort group of five animals was used to test the effect of Freund's adjuvant on lowering the primary irritation determined in the preliminary tests. Five per cent of the test substance (corresponding to 2.9% of water-free substance) was used for the intradermal induction phase, followed by an epidermal induction on day 9 and epidermal challenge on day 22 with the undiluted test substance (corresponding to 57.9% water-free test substance). The treated animals showed no clinical signs during the study. Intradermal injections with Freund's adjuvant caused only very slight erythema and oedema. The treated skin was dry-chapped, developed fine and coarse scales, was light encrusted, was indurated and had white lumps and necrosis. Following the challenge application, 24 and 48 hours after removal of the occlusive bandage, the animals showed no effects (Schollmeier & Leist, 1989c).

Figure 4. The proposed metabolic pathway of NAG in rats

Deacetylation
mainly in the intestine
(reversible)

disodium N-acetyl -L-glufosinate
(L-NAG, AE F099730)

L-glufosinate - free acid, AE F057740
(D,L-GA, glufosinate ammonium:
AE F039866)

Oxidative
Deamination

4-methylphosphinico-2-oxo-
butanoic acid
(PPO, AE F065594)
[transient]

2-hydroxy-4-methylphosphinico-
butanoic acid
(MHB, AE F053705)
(disodium salt: AE F042231)

Decarboxylation

4-methyl-phosphinico-butanoic acid
(MPB, AE F039046)

3-methylphosphinico-propionic acid
(MPP, AE F061517)

Oxidation,
Decarboxylation

2-methylphosphinico acetic acid
(MPA, AE F064619)

Table 27. Results of studies of acute toxicity with NAG

Species	Strain	Sex	Route	Vehicle	Purity (%)	LD_{50} (mg/kg bw)	Reference
Mouse	NMRKf (SPF71)	M+F	Oral	Deionized water	95.9	> 2895	Schollmeier & Leist (1989a)[a]
Rat	WISKf(SPF71)	M+F	Oral	Deionized water	82.6	> 2895	Schollmeier & Leist (1989b)[a]

F, female; LD_{50}, median lethal dose; M, male

[a] Performed according to GLP and OECD Test Guideline No. 401 using NAG (purity 82.6%) supplied as an aqueous solution with a water content of 42.1%. Presented NAG doses are corrected for water content in the aqueous solution.

5.2 *Short-term studies of toxicity*

Mice

NMRI mice (five of each sex per group) were fed diets containing NAG (purity 79.4%, delivered in aqueous solution with water content of 42.1%, active ingredient content 57.9%) for 4 weeks at a nominal concentration of 0, 116, 579, 2895 or 5790 ppm (equal to average concentrations of 0, 21.7, 124, 600 and 1126 mg/kg bw per day for males and 0, 22.9, 115, 591 and 1148 mg/kg bw per day for females, respectively). The study was performed in accordance with OECD Test Guideline No. 407. Mice were examined daily. Body weights and feed consumption were recorded twice weekly, and water consumption was recorded once weekly. Animals were sacrificed at the end of the 4-week treatment period. Haematological examination, clinical chemistry and urine analysis were carried out at the termination of the study. In addition, the activity of glutamine synthetase was examined in liver and brain. At necropsy, animals were examined macroscopically and histologically. Statements of adherence to QA and GLP were included.

During the study, behaviour and general health conditions were unaffected. No signs of neurological disturbances, opacity of the refracting media of the eyes, lesions of the oral mucosa or disturbances of dental growth were observed in any of the treated groups. No clinical signs of systemic toxicity (specifically indicative of central nervous system intoxication) were observed, and no animals died during the study. No changes in body weight gain or feed and water consumption were reported.

There were a few statistically significant changes in the haematology and clinical chemistry parameters, without any toxicological significance, apart from the slight decrease in calcium levels in females from 2895 ppm (equal to 591 mg/kg bw per day) and higher, for which substance dependency cannot be excluded. Determination of glutamine synthetase activity revealed a significant inhibition in the liver (21–45%) and brain (37–43%) at 2895 and 5790 ppm (Table 28). A 14% reduction in brain synthetase activity in females at 579 ppm did not reach statistical significance.

Table 28. Effect of treatment with NAG on glutamine synthetase activity in mice

Organ	Sex	Glutamine synthetase activity (± SD) (nkat/mg protein)				
		0 ppm	116 ppm	579 ppm	2895 ppm	5790 ppm
Liver	Male	0.43 (± 0.06)	0.38 (± 0.03)	0.38 (± 0.03)	0.34* (± 0.04)	0.24* (± 0.04)
	Female	0.38 (± 0.04)	0.50* (± 0.06)	0.40 (± 0.03)	0.42 (± 0.05)	0.22* (± 0.02)
Brain	Male	1.24 (± 0.11)	1.44 (± 0.10)	1.52 (± 0.09)	1.12 (± 0.16)	0.71* (± 0.12)
	Female	1.47 (± 0.09)	1.41 (± 0.20)	1.26 (± 0.17)	0.92* (± 0.14)	0.89* (± 0.15)

From Ebert (1991a)
SD, standard deviation
* $P < 0.05$ (according to the parametric method of Dunnett)

The effect on glutamine synthetase activity was considered at the time the report was issued to be due to the contamination of the test article with glufosinate (4.5%), a proven inhibitor of glutamine synthetase. However, subsequent rat metabolism work shows that glutamine synthetase inhibition would also be expected as a result of glufosinate formed by in vivo deacetylation of NAG to glufosinate. The combination of test substance impurity and deacetylation provides the full explanation for the level of glutamine synthetase inhibition observed. Urine analysis showed no treatment-related changes in any of the groups. Analysis of organ weights indicated no substance-related changes in any group. There were no treatment-related macroscopic or microscopic changes.

The NOAEL was 579 ppm (equal to 115 mg/kg bw per day), based on a significant reduction (37%) in brain glutamine synthetase activity at 2895 ppm (equal to 591 mg/kg bw per day) (Ebert, 1991a).

A subchronic (13-week) oral feeding study was performed in mice, according to OECD Test Guideline No. 408. SPF-bred Hanover-derived NMRI mice (20 animals of each sex per group) were treated via diet with NAG (purity of technical ingredient 74.7%) in the diet. Dietary concentration levels were 0, 500, 2000 and 8000 ppm. Actual dose levels were 0, 83, 233 and 1296 mg/kg bw per day for males and 0, 110, 436 and 1743 mg/kg bw per day for females, respectively. Mice were examined at least once daily. Detailed clinical observations (including palpation for tissue mass) were done once a week. Body weights and feed consumption were recorded weekly. Blood and urine sampling was performed at week 13. Animals were sacrificed at the end of the 13-week treatment period. At necropsy, animals were examined macroscopically and histologically. In addition, the activity of glutamine synthetase was examined in liver, kidney and brain samples. Statements of adherence to QA and GLP were included.

One male died at 8000 ppm after blood sampling in week 13, but this was unrelated to treatment. No treatment-related clinical signs were observed. Body weight development and the absolute and relative feed intake were not affected. Blood analysis showed a statistically significant increase of lactate dehydrogenase in two high-dose males only. This effect was not observed in females, the values were within the normal historical control range and this effect was not seen in the 28-day mouse study and therefore not considered to be of biological significance. Glutamine synthetase activity was significantly inhibited in liver, kidney and brain (Table 29).

Table 29. Effect of NAG on glutamine synthetase activity in liver, kidney and brain of mice

Organ	Sex	Glutamine synthetase activity[a]			
		0 ppm	500 ppm	2000 ppm	8000 ppm
Liver	Male	3.98	3.74	3.34**	2.94**
		—	(94)	(84)	(74)
	Female	4.97	4.91	4.60*	3.98**
		—	(99)	(93)	(80)
Kidney	Male	1.42	1.01**	0.83**	0.71**
		—	(71)	(58)	(50)
	Female	1.73	1.29**	1.20**	1.10**
		—	(75)	(69)	(64)
Brain	Male	3.57	3.32**	3.17**	2.59**
		—	(93)	(89)	(73)
	Female	3.38	3.32	2.94**	2.18**
		—	(98)	(87)	(64)

From Tennekes, Schmid & Probst (1992)

* $P < 0.05$; ** $P < 0.01$ (according to the parametric method of Dunnett)

[a] Units of glutamine synthetase activity are micromoles of γ-glutamyl-hydroxamate formed per millilitre of reaction mixture per 20 minutes at 37 °C. Percentage of control value given in parentheses.

In the liver, slight to moderate inhibition of glutamine synthetase activity was seen at 2000 and 8000 ppm. In the kidney, slight to moderate inhibition was seen at all doses. In brain, glutamine synthetase activity was dose-dependently inhibited at 2000 and 10 000 ppm.

No treatment-related changes were seen in organ weights, necropsy findings or microscopic findings.

The NOAEL was 500 ppm (equal to 83 mg/kg bw per day), based on the inhibition of brain glutamine synthetase activity (11–13%) at 2000 ppm (equal to 233 mg/kg bw per day) (Tennekes, Schmid & Probst, 1992).

Rats

Wistar rats (five of each sex per group) were fed diets containing NAG (purity 79.4%, delivered in aqueous solution with water content of 42.1%, active ingredient content 57.9%) for 4 weeks at a nominal concentration of 0, 116, 579, 2895 or 5790 ppm (equal to 0, 12.1, 59.9, 309 and 593 mg/kg bw per day for males and 0, 11.2, 55.1, 283 and 561 mg/kg bw per day for females, respectively). The study was performed in accordance with OECD Test Guideline No. 407. Rats were examined daily. Body weights and feed consumption were recorded twice weekly, and water consumption was recorded once weekly. Animals were sacrificed at the end of the 4-week treatment period. Haematological examination, clinical chemistry and urine analysis were carried out at the termination of the study. In addition, the activity of glutamine synthetase was examined in liver and brain. At necropsy, animals were examined macroscopically and histologically. Statements of adherence to QA and GLP were included.

During the study, behaviour and general health conditions were unaffected. No signs of neurological disturbances, opacity of the refracting media of the eyes, lesions of the oral mucosa or disturbances of dental growth were observed in any of the treated groups. No clinical signs of systemic toxicity (specifically indicative of central nervous system intoxication) were observed, and no animals died during the study. No changes in body weight gain or feed and water consumption were reported.

There were a number of statistically significant changes in the haematology and clinical chemistry parameters; however, most of them were considered to reflect normal biological variation and were not caused by the test substance. However, a treatment-related effect cannot be excluded for lactate dehydrogenase, for which there was a statistically significant decrease at the high dose level in females. However, as this possible effect was a decrease in the enzyme, it is not of toxicological significance. Determination of glutamine synthetase activity revealed a significant inhibition (about 50%) in the livers of the animals of both sexes at the high dose level. This effect was observed at a dose level of 579 ppm and above in males and at 2895 ppm and above in females. Statistically significant decreases in glutamine synthetase (up to 39%) were found in the brain at the three highest dose levels in females and at 116 and 2895 ppm in males. However, the decreases showed no dose–response relationship. The effect on glutamine synthetase activity was considered at the time the report was issued to be due to the contamination of the test article with glufosinate (4.5%), a proven inhibitor of glutamine synthetase. However, subsequent rat metabolism work shows that glutamine synthetase inhibition would also be expected as a result of glufosinate formed by in vivo deacetylation of NAG to glufosinate. The combination of test substance impurity and deacetylation could explain the level of glutamine synthetase inhibition observed. In a 13-week dietary study by Schmid et al. (1998) with NAG containing glufosinate as an impurity at 0.1%, no effect on brain glutamine synthetase activity was found. Thus, the Meeting concluded that it is likely that the observed effects on brain synthetase in the present study can be attributed to the presence of glufosinate-ammonium as an impurity of NAG and the deacetylation of NAG to glufosinate. Urine analysis showed no treatment-related changes in any of the groups. Analysis of organ weights indicated a slight decrease in absolute and relative heart weights among the males from all dose groups, reaching statistical significance for absolute heart weight in the highest-dose group. As the effect was observed in all treatment groups and was not dose dependent and as no histopathological changes in the heart were observed, the Meeting considered this a fortuitous finding. No other changes that might be related to the test substance were detected. There were no treatment-related macroscopic or microscopic changes.

The NOAEL was 5790 ppm (equal to 561 mg/kg bw per day), the highest dose tested (Ebert, 1991b).

A subchronic (13-week) oral feeding study was performed in rats, according to OECD Test Guideline No. 408. SPF-bred Hanover-derived Wistar rats (10 animals of each sex per group and 10 animals of each sex in the control, mid-dose and high-dose groups for recovery) were treated via diet containing NAG (purity of technical ingredient 79.4%, supplied as a 44% w/w solution in water).

Dietary concentration levels were 0, 400, 2000 and 10 000 ppm, corresponding to 0, 900, 4500 and 22 500 mg test substance per kilogram diet. Actual dose levels were 0, 29.1, 147 and 738 mg/kg bw per day for males and 0, 31.7, 162 and 800 mg/kg bw per day for females, respectively. Rats were examined at least once daily. Detailed clinical observations (including palpation for tissue mass) were done once a week. Body weights and feed consumption were recorded weekly. Ophthalmoscopic examinations were performed on all animals pretest and at 11 and 16 weeks. Blood and urine sampling was performed at 13 and 17 weeks. Animals were sacrificed at the end of the 13-week treatment period or after recovery (17 weeks). At necropsy, animals were examined macroscopically and histologically. In addition, the activity of glutamine synthetase was examined in liver, kidney and brain samples. Statements of adherence to QA and GLP were included.

There were no unscheduled deaths during the study, and no treatment-related clinical signs were observed. Body weight development and absolute and relative feed intake were not affected. Very slight, but statistically significant, decreases in erythrocyte count (4%) and haemoglobin concentration (3%), a marginal increase in corpuscular volume index (3%) for males and a very slight increase in prothrombin time (2%) in males at 10 000 ppm were observed. No biological significance was attributed to these findings, as they were all marginally different from the control group values and within the range of the historical control data. Furthermore, at the end of the recovery period, these findings were no longer statistically different from the control values. For clinical chemistry, glutamine synthetase inhibition was observed in liver, kidney and brain tissues (Table 30).

In liver of high-dose males, glutamine synthetase activity was inhibited to 47% of control values. In males and females of the high-dose group, brain glutamine synthetase activity was decreased to 88–89% of control activity. After the recovery period, slight inhibition (6–8%) was still observed in the liver of females and in the brain (6–8%) of both sexes at 10 000 ppm. Statistically significantly higher kidney weights were observed in males at 400 and 10 000 ppm at 13 weeks. As no statistically significant increase was observed at the middle dose and there were no histopathological effects on the kidneys, they were considered not to be of toxicological significance. No effects were observed after the recovery period. There were no treatment-related necropsy findings or microscopic findings.

The NOAEL was 2000 ppm (equal to 147 mg/kg bw per day), based on statistically significant inhibition (11–12%) of brain glutamine synthetase activity at 10 000 ppm (equal to 738 mg/kg bw per day) (Tennekes, Probst & Luetkemeier, 1992).

Dogs

A subchronic (13-week) oral feeding study was performed in dogs, according to OECD Test Guideline No. 409. Purebred Beagles (four [group 2] or six [groups 1, 3 and 4] animals of each sex per group) were treated with NAG (purity of technical ingredient 74.7%) in the diet. Dietary concentration levels were 0, 500, 2000 and 8000 ppm, corresponding to an average test article intake of 0, 20, 76 and 294 mg/kg bw per day, respectively. Dogs were examined twice daily for viability and clinical signs. Body weights were recorded weekly, and feed consumption was recorded daily. Ophthalmoscopic examinations were performed 4 times (pretest and at 4 weeks, 13 weeks and 4 weeks after recovery), as was blood and urine sampling. Animals were sacrificed at the end of the 13-week treatment period or after the 4-week recovery period. At necropsy, animals were examined macroscopically and histologically. In addition, the activity of glutamine synthetase was examined in liver, kidney and brain samples. Statements of adherence to QA and GLP were included.

All animals survived to the end of the scheduled treatment or recovery periods, and no clinical signs were observed. Feed consumption and body weight were unaffected, and there were no treatment-related ophthalmoscopic changes. There were no treatment-related haematological findings. The plasma clinical biochemistry parameters were unaffected by treatment. A moderate to marked, dose-dependent inhibition of glutamine synthetase activity was observed in the liver (43–79%) and brain (16–65%) taken after 13 weeks of treatment from dogs at 2000 or 8000 ppm (Table 31).

Table 30. Efect of NAG on glutamine synthetase activity in liver, kidney and brain of rats

Organ	Sex	Sampling week	Glutamine synthetase activity[a]			
			0 ppm	400 ppm	2000 ppm	10 000 ppm
Liver	Male	13	3.76[a]	2.76**	2.18**	1.77**
			—	(73)	(58)	(47)
		17	3.17	—	3.49	3.42
			—	—	(110)	(108)
	Female	13	3.71	3.12*	2.57**	2.35**
			—	(84)	(69)	(63)
		17	3.73	—	3.53	3.32 *
			—	—	(95)	(89)
Kidney	Male	13	2.10	1.68**	1.49**	1.64**
			—	(80)	(71)	(78)
		17	2.13	—	2.09	1.88
			—	—	(98)	(88)
	Female	13	1.17	1.12	1.20	1.52**
			—	(96)	(103)	(130)
		17	1.27	—	1.28	1.32
			—	—	(101)	(104)
Brain	Male	13	3.19	3.26	3.02*	2.82**
			—	(102)	(95)	(88)
		17	3.14	—	2.98	2.89**
			—	—	(95)	(92)
	Female	13	3.12	3.06	3.02	2.77*
			—	(98)	(97)	(89)
		17	3.09	—	3.11	2.92*
			—	—	(101)	(94)

From Tennekes, Probst & Luetkemeier (1992)
* $P < 0.05$; ** $P < 0.01$ (according to the parametric method of Dunnett)
[a] Units of glutamine synthetase activity are micromoles of γ-glutamyl-hydroxamate formed per millilitre of reaction mixture per 20 minutes at 37 °C. Percentage of control value given in parentheses.

A slight, statistically significant decrease (31%) in liver glutamine synthetase activity was also seen in males at 500 ppm. Glutamine synthetase activity in the kidney was unaffected by treatment at all dose levels. Following 4 weeks of recovery, no statistically significant difference between control and test groups was observed. Urine analysis showed lower specific gravity and osmolality in females at 8000 ppm after 4 and 13 weeks of treatment and at the end of the recovery period. In the absence of histopathological changes in the kidney, these findings were not considered to be of toxicological relevance. No treatment-related changes were seen in organ weights, necropsy findings or microscopic findings.

The NOAEL was 500 ppm (equal to 20 mg/kg bw per day), based on reduction in brain glutamine synthetase activity (≥ 16%) at 2000 ppm (equal to 76 mg/kg bw per day) (Corney, Braunhofer & Luetkemeier, 1992).

Table 31. Effect of NAG on glutamine synthetase activity in liver, kidney and brain areas of dogs

Organ	Sex	Sampling week	Glutamine synthetase activity[a]			
			0 ppm	500 ppm	2000 ppm	8000 ppm
Liver	Male	13	2.72	1.88**	1.15**	0.58**
		17	2.61	—	2.39	2.03
	Female	13	1.85	1.63	1.05*	0.68**
		17	2.06	—	1.72	1.26
Kidney	Male	13	0.02	0.04	0.06	0.05
		17	0.07	—	0.05	0.06
	Female	13	0.04	0.10	0.06	0.09
		17	0.05	—	0.08	0.07
Brain (cortex)	Male	13	3.17	3.35	2.82	2.30*
		17	2.97	—	2.40	2.49
	Female	13	2.90	3.15	2.70	2.48
		17	3.11	—	2.74	2.35
Brain (midbrain)	Male	13	2.33	2.62	2.58	1.37*
		17	2.10	—	2.29	2.12
	Female	13	2.71	2.23	2.39	2.04
		17	2.09	—	2.07	1.83
Brain (cerebellum)	Male	13	1.58	1.42	1.26*	0.85**
		17	1.42	—	1.23	1.20
	Female	13	1.59	1.47	1.34**	0.87**
		17	1.49	—	1.30	1.14
Brain (brainstem)	Male	13	1.36	1.30	0.90**	0.47**
		17	1.32	—	0.95	0.93
	Female	13	1.20	1.19	0.91**	0.73**
		17	1.35	—	1.17	0.83

From Corney, Braunhofer & Luetkemeier (1992)
* $P < 0.05$; ** $P < 0.01$ (according to the parametric method of Dunnett)
[a] Units of glutamine synthetase activity are micromoles of γ-glutamyl-hydroxamate formed per millilitre of reaction mixture per 20 minutes at 37 °C.

A subchronic (52-week) oral dietary toxicity study was performed in dogs, according to OECD Test Guideline No. 452. Purebred Beagles (six animals of each sex per group) were treated with NAG (purity 92.4%, supplied as 35.7% solution in water) in the diet. Dietary concentrations were 0, 100, 1000 and 8000 ppm, corresponding to an average test article intake of 0, 4.0, 44 and 325 mg active ingredient per kilogram body weight per day for males and 0, 4.3, 43 and 346 mg active ingredient per kilogram body weight per day for females, respectively. Dogs were examined twice daily for viability and clinical signs and were physically examined once a week. Body weights were recorded weekly, and feed consumption was recorded daily. Ophthalmoscopic examinations were performed 3 times: at weeks 12, 25 and 51 of treatment. Prior to the start of treatment and in weeks 13, 26 and 52, blood was collected for haematology and clinical chemistry evaluation. Urine was collected in the same weeks for urine analysis. Two animals of each sex from each dose level were sacrificed after 26 weeks of treatment (at 2000 ppm only one female, because one female had already been killed in week 3). All remaining animals were killed after 52 weeks of treatment. Gross and histopathological examinations were performed on all animals. Statements of adherence to QA and GLP were included.

One female (mid-dose group) was killed in extremis during week 3 of treatment. Microscopic examination revealed a non-suppurative encephalitis of unknown origin, consistent with the clinically observed convulsions. Etiology could not be established, but a post-vaccine reaction cannot be excluded. In males receiving 1000 or 8000 ppm, soft faeces were observed more frequently. Feed consumption and body weight were unaffected, and there were no treatment-related ophthalmoscopic changes. There were no treatment-related haematological findings. Small, but statistically significant, increases (differential lymphocyte count) or decreases (haematocrit and monocyte count) were occasionally observed, but were not found to be dose related and therefore were considered to be incidental. There were no changes in the plasma clinical biochemistry parameters. After weeks 13, 26 and 52, decreased lactate dehydrogenase values were observed in high-dose animals compared with controls. Lower lactate dehydrogenase values (50%) were also seen in mid-dose females (pretreatment and at weeks 26 and 52). As these decreases were not associated with any other changes and because decreased mean lactate dehydrogenase values were initially seen pretreatment in mid- and high-dose females, these differences were considered to be of no toxicological significance. Small and transient changes were seen in creatinine, creatine phosphokinase, glucose, blood urea nitrogen and chloride, but these were inconsistent between sampling occasions. Likewise, the transient increases in ALP and ALT levels observed in week 26 in a low-dose male and a high-dose female were considered incidental. Urine analysis showed no treatment-related changes. Decreased absolute and relative spleen weights were observed in males at 1000 and 8000 ppm after 12 months. The changes were not clearly dose related, and no gross pathological or histopathological findings were reported, and they were therefore considered not to be of toxicological significance. Glutamine synthetase activity was not determined in this study.

The NOAEL was 8000 ppm (equal to 325 mg/kg bw per day), the highest dose tested (Bernier, 1996).

5.3 Long-term studies of toxicity and carcinogenicity

Mice

In a 2-year dietary carcinogenicity study, technical NAG (purity 92.43%, supplied as a 35.7% solution in water) was administered to groups of 70 male and 70 female CRL:CD mice at 0, 100, 1000 or 8000 ppm (equal to 0, 15, 148 and 1188 mg/kg bw per day for males and 0, 19, 187 and 1460 mg/kg bw per day for females, respectively). An additional 20 mice of each sex per group were designated for interim sacrifice after 52 weeks of treatment. The mice were checked daily for mortality and clinical signs. A detailed clinical examination and palpation for nodules and masses were performed weekly. Feed consumption and body weight were recorded weekly during months 1–3 and biweekly thereafter. Blood was collected after 52 weeks (interim group) and 78 and 104 weeks of treatment in the carcinogenicity group. Glutathione levels in whole blood and liver tissue were measured in the remaining fasted animals at termination of the study. At termination at 104 weeks, all surviving animals were killed and organs were weighed. Gross pathological examination was performed for all animals. Histopathological examinations were performed on all control and high-dose animals, on low- and intermediate-dose animals that were found dead or were killed before the termination of the study, on the lungs, kidneys, liver and adrenal glands in all low- and intermediate-dose animals and on any gross lesions observed macroscopically for animals in all dose groups. Statements of adherence to QA and GLP were included.

The survival rates of control and treated animals were similar. No treatment-related clinical signs were observed. No treatment-related effects were seen on feed consumption. There were no treatment-related effects on haematology or on the clinical biochemistry values of the interim sacrifice or carcinogenicity animals when compared with the control group. At termination of the study, statistically significant increases in total protein and globulin levels were seen in males treated at 10 000 ppm. These increases were not considered to be of toxicological significance, as there was no evidence of an effect at this dose level, nor was there a correlation with any histopathological finding. There was no effect on organ weights. There were no macroscopic or microscopic neoplastic or non-neoplastic findings in either the interim sacrifice or carcinogenicity group animals that could be associated with the administration of the test substance. The Meeting noted that salivary gland

amyloidosis tended to be dose-dependently increased in females. However, such an increase was not observed in males. It was further noted that amyloidosis occurred frequently in other organs in males and females of all dose groups, generally without dose dependency. The Meeting therefore considered the increased incidence of amyloidosis in salivary glands in females to be a fortuitous finding.

The NOAEL was 8000 ppm (equal to 1188 mg/kg bw per day), the highest dose tested (Farrell, 1997; Ernst & Stumpf, 1999a).

Rats

A 52/104-week combined toxicity and carcinogenicity study was performed on Sprague-Dawley Crl:CDR(SD)BR rats (100 of each sex per dose). Ten rats of each sex per group were killed after 52 weeks of treatment, and 70 rats of each sex per group after 104 weeks; 20 rats of each sex per group were used as satellite groups. The groups of rats were fed diet with NAG (purity not given, supplied as a 35.7% solution) at a technical dose level (dry portion of the material) of 0, 200, 2000 or 20 000 ppm (equal to 0, 9, 91 and 998 mg/kg bw per day for males and 0, 11, 108 and 1212 mg/kg bw per day for females of the carcinogenicity groups [104 weeks]). No separate calculations were reported for the toxicity groups (52 weeks). The rats were examined daily for mortality and clinical signs. Additionally, a detailed clinical examination was performed weekly. Body weight and feed consumption were recorded weekly for the first 3 months and every 2 months thereafter. Ophthalmoscopic evaluations were performed on all animals in the pretreatment period and additionally in week 50 (toxicity subgroup) and week 101 (satellite subgroup). Haematology, clinical chemistry and urine analysis were performed at weeks 25 and 51 (10 animals of each sex per dose from the toxicity subgroup and 10 animals of each sex per dose from the satellite subgroup) and during weeks 78 and 102 on the surviving animals in the satellite subgroups. No additional glutamine synthetase measurements were performed. Weights of adrenals, brain, heart, kidneys, liver, ovaries, pituitary, spleen, testes and thyroid were determined at scheduled necropsy from each animal euthanized after 52 weeks. All remaining animals were sacrificed after 104 weeks of treatment. Gross pathological examination was performed for all animals, and histopathological examination was performed on the control and high-dose animals of the carcinogenicity, toxicity and satellite subgroups, regardless of mode of death. In addition, histopathology was performed on all animals that died or were killed in extremis during the conduct of the study and on any gross lesions observed macroscopically for animals in the low- and mid-dose groups. Macroscopic and microscopic examinations of a wide range of organs and tissues were performed on all animals. Statements of adherence to QA and GLP were included.

There were no significant differences in mortality between treatment and control groups of the chronic toxicity animals for the major duration of the study. A high incidence of soft faeces was noted at 20 000 ppm in both sexes after approximately 8 weeks. Lower body weight gains were observed in both sexes at 20 000 ppm (about 10%), although feed consumption was consistently higher throughout the first 88 (males) or 68 weeks (females) than that of controls. There was no evidence that treatment resulted in any ophthalmological changes. At week 102, statistically significantly increased haematocrit values and mean cellular volumes as well as decreased mean haemoglobin concentrations were seen in the mid- and high-dose females (Table 32). However, these changes were of small magnitude, and the values were within the normal physiological range for rats of this age and strain.

Other statistically significant intergroup differences observed in clinical chemistry parameters were considered not to be treatment related, because the changes were within the normal physiological range for rats of this age and strain, did not show a dose–response relationship or were inconsistent between sampling occasions. Evaluation of the urine analysis results indicated no treatment-related effects. Evaluation of the organ weight data obtained from animals sacrificed after 52 weeks of treatment revealed a slight increase in kidney weights (absolute, relative to body weight and to brain weight, 14–21%) in females at 20 000 ppm. An increased incidence, statistically significant, of renal chronic progressive nephropathy was seen in female rats killed after 52 weeks of treatment at 20 000 ppm in the carcinogenicity subgroup (41/70) compared with controls (16/70).

However, chronic progressive nephropathy is a kidney alteration that occurs spontaneously in ageing rats and is not considered relevant for humans. A dose-related statistically significant difference was seen in the incidence of urolithiasis in females at 20 000 ppm in the carcinogenicity and satellite subgroups. In the high-dose animals (main plus satellite groups), there was an increased incidence of polyarteritis nodosa in testes (18/90 [20%] in controls; 33/90 [37%] at 20 000 ppm, historical control range 16–31%) and blood vessels (males: 4/7 in controls; 11/18 at 20 000 ppm; females: 4/4 in controls; 9/11 at 20 000 ppm; no historical control data). Although within the historical control ranges (8–46% for males; 15–36% for females), an increased incidence of extramedullary haematopoiesis (males: 12/90 [13%] in controls; 25/90 [28%] at 20 000 ppm; females: 10/90 [11%] in controls; 28/90 [31%] at 20 000 ppm) was observed.

Table 32. Effects seen in rats exposed to NAG in the diet

Parameter	Week	Mean value							
		Males				Females			
		0 ppm	200 ppm	2000 ppm	20 000 ppm	0 ppm	200 ppm	2000 ppm	20 000 ppm
Haematocrit (%)	25	42.4	41.9	41.9	40.6**	41.3	41.4	41.5	39.9*
	51	40.6	40.5	40.0	39.4	40.2	39.3	39.7	38.7
	78	39.9	39.7	40.1	39.7	39.1	38.9	36.4	36.6
	102	33.6	35.3	33.5	36.9	36.2	42.5	55.7***	49.4*
Mean cell volume (µm^3)	25	50.1	50.2	49.8	49.7	53.9	53.7	53.3	53.5
	51	51.3	51.3	51.0	51.0	54.9	54.6	54.8	55.0
	78	52.7	52.7	52.7	52.4	55.7	54.6	56.0	56.0
	102	55.3	51.3	54.3	53.8	57.4	64.3	79.3***	82.1***
Mean cell haemoglobin concentration (pg)	25	36.2	36.7	36.8*	37.0**	36.1	36.4	36.7*	37.2**
	51	36.6	36.5	36.8	36.9	36.6	36.7	37.0*	37.1**
	78	36.3	36.3	36.4	36.3	36.2	36.7*	36.9**	36.4
	102	36.4	36.6	36.5	36.8	36.8	33.7	25.5***	25.1***

From Bernier (1997); Ernst & Stumpf (1999b); Chevalier (2001)
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Analysis of the tumour data did not reveal any biologically significant differences in the type or distribution of tumours observed in controls or treated animals.

The NOAEL was 2000 ppm (equal to 91 mg/kg bw per day), based on decreased body weight gain, increased incidence of soft faeces and increased incidences of poliarteritis nodosa in blood vessels and testes and urolithiasis at 20 000 ppm (equal to 998 mg/kg bw per day) (Bernier, 1997; Ernst and Stumpf, 1999b; Chevalier, 2001).

5.4 Genotoxicity

NAG was tested for genotoxicity in a range of guideline-compliant assays. No evidence for genotoxicity was observed in any test.

The results of the genotoxicity tests are summarized in Table 33.

Table 33. Overview of genotoxicity tests with NAG

End-point	Test object	Concentration	Purity (%)	Results	Reference
In vitro					
Point mutations	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538; *Escherichia coli* WP2 *uvrA*	2.33–2910 µg/plate (±S9)	82.6	Negative	Müller (1989a)[a]
Chromosomal aberrations	V79 Chinese hamster ovary cells	29–1546.5 µg/ml (±S9, cytotoxicity), 154.6, 773.5 and 1546.5 µg/ml (±S9, chromosomal aberration study)	79.4	Negative	Müller (1989b)[b]
Chromosomal aberrations	Human lymphocytes	600, 3000 and 5000 µg/ml (−S9, 24 h), 5000 µg/ml (−S9, 28 h), 600, 3000, 4750 µg/ml (+S9, 24 h), 4750 µg/ml (+S9, 48 h)	74.7	Negative	Heidemann & Voelkner (1992)[c]
Gene mutation	V79 Chinese hamster lung cells, HGPRT test	1000, 1500, 2000, 2671 µg/ml (±S9), equivalent to 582, 873, 1164 and 1555 µg/ml on a water-free basis	82.6	Negative	Müller (1989c)[d]
Gene mutation	V79 Chinese hamster lung cells, HGPRT test	1000, 1500, 2000, 2671 µg/ml (±S9), equivalent to 444, 666, 888 and 1186 µg/ml on a water-free basis	74.7	Negative	Müller (1991a)[e]
Unscheduled DNA synthesis	Human cell line A549	0.582–582 µg/ml (±S9)	79.4/82.6	Negative	Müller (1989d)[f]
Unscheduled DNA synthesis	Human cell line A549	3–3000 µg/ml (±S9), equivalent to 1.332–1332 µg/ml on a water-free basis	74.7	Negative	Müller (1991b)[g]
In vivo					
Micronucleus	NMRI mouse (5/sex/dose/time point) (3/sex/dose in preliminary dose range–finding study)	0, 500, 2500 and 5000 mg/kg bw (single gavage doses of technical substance) at each time point (24, 48 and 72 h after administration), equivalent to 0, 222, 1100 and 2220 mg/kg bw on a water-free basis	74.7	Negative	Müller (1991c)[h]

DNA, deoxyribonucleic acid; S9, 9000 × *g* supernatant fraction of rat liver homogenate

[a] Performed in accordance with OECD Test Guideline No. 471. Dose levels selected based on absence of toxicity in a toxicity test with dose levels up to 5820 µg/plate. Positive controls were sodium azide, 9-aminoacridine, 2-nitrofluorene and *N*-methyl-*N*-nitroguanidine in the absence of S9 and benzo[*a*]pyrene and 2-aminoanthracene in the presence of S9.

Table 33 (continued)

[b] Performed in accordance with OECD Test Guideline No. 473. Positive controls used were ethylmethanesulfonate (−S9) and cyclophosphamide (Endoxan) (+S9). Two hundred cells per plate were analysed. NAG induced a significant increase in the aberration rate, but only in cells inclusive with gaps 7 hours after treatment with 1546.5 μg/ml with metabolic activation (S9 mix). Also at the preparation time of 18 hours without S9 mix, all three concentrations produced a very slight increase in the number of aberrations in cells inclusive of gaps. Additionally, the number of cells with aberrations inclusive of gaps was very slightly increased at the 28-hour preparation time at the highest dose level with metabolic activation. The observed aberrations at the 18-hour and 28-hour preparation times were considered of no relevance because of the very slight increase in the numbers and the fact that solvent control values were lower than usual.

[c] Performed in accordance with OECD Test Guideline No. 473. Positive controls used were ethylmethanesulfonate (−S9) and cyclophosphamide (+S9). For each culture, 100 metaphases were scored for structural chromosomal aberrations (positive control 25 metaphases). Treatment of the cells even with the highest concentration (5.00 mg/ml) did not reduce the mitotic indices at both fixation intervals in either the presence or absence of S9 mix. In both experiments, there was no biologically relevant increase in cells with aberrations after treatment with the test article at any fixation intervals.

[d] Performed in accordance with OECD Test Guideline No. 476. NAG was supplied as an aqueous solution (water content 41.8%). Positive controls used were ethylmethanesulfonate (−S9) and 9,10-dimethyl-1,2-benzanthracene (+S9). The test substance produced no significant cytotoxic effect from 50 μg/ml up to the highest concentration of 2671 μg/ml. The test compound did not induce a significant increase in the number of mutant colonies or in the mutation frequency at any dose level of the test substance either with or without S9 mix. No cytotoxic effect was observed in the main experiment. Marked increases in mutation frequency were obtained with the positive control substances.

[e] Performed in accordance with OECD Test Guideline No. 476. NAG was supplied as an aqueous solution (water content 55.6%). Positive controls used were ethylmethanesulfonate (−S9) and 9,10-dimethyl-1,2-benzanthracene (+S9). The test substance produced no significant cytotoxic effect from 50 μg/ml up to the highest concentration of 2671 μg/ml (10 mmol/l). No increase in mutant colony numbers was obtained at any dose level in the two independent experiments. The positive control substances showed an increase in induced mutant colonies.

[f] Performed in accordance with OECD Test Guideline No. 482. NAG was supplied as an aqueous solution (water content 42.1%/41.8%). Positive controls used were 4-nitroquinoline-*N*-oxide (−S9) and benzo[*a*]pyrene (+S9). No cytotoxicity and no increase in unscheduled DNA synthesis were observed at any of the test substance concentrations in either the presence or absence of S9 mix. A significant induction of unscheduled DNA synthesis was obtained with the positive control substances.

[g] Performed in accordance with OECD Test Guideline No. 482. NAG was supplied as an aqueous solution (water content 55.6%). Positive controls used were 4-nitroquinoline-*N*-oxide (−S9) and benzo[*a*]pyrene (+S9). No cytotoxicity and no increase in unscheduled DNA synthesis were observed at any of the test substance concentrations in either the presence or absence of S9 mix. A significant induction of unscheduled DNA synthesis was obtained with the positive control substances.

[h] Study design resembles OECD Test Guideline No. 474. NAG was supplied as an aqueous solution (water content 55.6%). The number of polychromatic and normochromatic erythrocytes containing micronuclei was not increased. The ratio of polychromatic to normochromatic erythrocytes in both male and female animals remained unaffected by the treatment with NAG and was not statistically different from the control values. The positive control used was cyclophosphamide (Endoxan). It induced a marked statistically significant increase in the number of polychromatic cells with micronuclei in both sexes, indicating the sensitivity of the system. The ratio of polychromatic erythrocytes to normocytes was not changed to a significant extent.

5.5 Reproductive and developmental toxicity

(a) Multigeneration studies

Rats

In a range-finding one-generation reproductive toxicity study, NAG was administered in the diet during a 3-week premating period and continuing throughout the mating, gestation and lactation periods to groups of 10 Sprague-Dawley rats of each sex per group. The purity was 92.4%. The raw test material had a water content of 64.3%. This was taken into account when calculating the dose level of technical material to be administered. Dietary concentrations were 0, 200, 2000 and 10 000 ppm (equal to 0, 13, 129 and 670 mg/kg bw per day for males and 0, 16, 156 and 799 mg/kg bw per

day for females, respectively). The rats were checked daily for mortality and clinical signs. Body weight and feed consumption were measured weekly. In dams that littered, body weight and feed consumption were measured on LDs 1, 4, 7, 14 and 21. Litter size, sex of pups, number of live and dead pups and malformations were recorded. Maternal rats were killed and necropsied at the end of lactation. The number of implantation sites was recorded. Ovaries, uterus, testes, epididymides and seminal vesicles of all parental animals were weighed. At the end of the lactation period, the pups were examined externally and killed. Statements of adherence to QA and GLP were included.

No mortality was observed. One mid-dose male was killed in a moribund state. His condition was considered not to be treatment related, as no high-dose animals showed similar clinical effects or gross changes to internal organs. Occasionally observed clinical signs occurred in all groups, including controls, and were considered not related to treatment. No significant reductions in body weight gain were observed. During the pretreatment and premating periods, feed consumption was increased in high-dose females. This finding was considered to be incidental. The organ weights, mating and fertility indexes, conception rate, duration of pregnancy, gestation index, number of implantations, post-implantation loss, sex ratio and number of live and dead pups were not affected by treatment. Parental animals showed no treatment-related gross changes. Pup viability, survival and lactation indices, clinical signs, body weights and macroscopic appearance in the treated groups were not different from controls (Beyrouty, 1996a).

In a two-generation dietary reproductive toxicity study, performed in accordance with OECD Test Guideline No. 416, Sprague-Dawley rats (30 of each sex per group for the F_0 generation, 26 of each sex per group for the F_1 generation) were fed NAG (purity 92.4%) at a dietary concentration of 0, 200, 2000 or 10 000 ppm. The corresponding NAG intakes during the different phases of the study are presented in Table 34.

Table 34. NAG intake in parental rats during different phases of the multigeneration reproductive toxicity study

Test group	Generation	Dietary concentration (ppm)	Test material intake (mg/kg bw per day)		
			Premating		Post-mating
			Males	Females	Females (gestation)
1	F_0	0	0	0	0
2		200	13	18	13
3		2000	137	173	126
4		10 000	702	891	622
1	F_1	0	0	0	0
2		200	16	19	12
3		2000	162	197	124
4		10 000	821	1008	652

From Beyrouty (1996b)

All adult animals were examined daily for clinical signs. Body weights were measured weekly for the males throughout the study and for the females during the premating period. For mated females, body weights during gestation and lactation were also recorded. Feed intake was measured during the premating period for both sexes, post-mating for males and during gestation for the mated females. Following up to 14 days of mating, the males and females were separated and the dams then allowed to litter. Pup clinical signs and body weights were recorded throughout lactation. On day 21 postpartum, the pups were weaned and sacrificed (except those forming the F_1 adult generation). The adults were killed soon after weaning of the pups, and each adult rat, together with one weanling of

each sex per litter, where possible, received a complete necropsy. For adult animals, organ weight measurements and epididymal and testicular sperm assessments were also conducted, and for adult rats in the control and high-dose groups, a histopathological examination was performed on a range of tissues. Statements of adherence to QA and GLP were included.

In the F_0 and F_1 adult generations, no treatment-related mortality occurred. Most high-dose males and some high-dose females showed brown fur staining (males only) and soft faeces. Males in the 2000 ppm group had a significantly ($P < 0.05$) reduced body weight at the end of week 1 when compared with the control group. Females in the 2000 ppm group had significantly ($P < 0.05$) lower body weights at the end of weeks 0 and 1. The body weights were also significantly ($P < 0.05$ or $P < 0.01$) reduced for the 200 ppm group females for weeks 1, 2 and 4. No significant differences were noted during gestation. Significant ($P < 0.05$) reductions in body weights were noted for the 200 ppm group females during lactation on days 0 and 21. These differences were considered not to be treatment related, as no significant differences were observed between the control and high-dose groups. In high-dose animals, feed consumption was occasionally slightly, but statistically significantly, increased during the premating period. Mating and fertility indices, gestational index, length of gestation, number of implantations, post-implantation loss, pup sex ratio, numbers of live or dead pups at birth and estrous cycle were not affected by treatment. Examination of sperm parameters, organ weights and gross and histological examination revealed no effect of treatment. A dose-related increase in the weight of the left seminal vesicles was seen in F_1 adults (up to 14%), reaching statistical significance in males at the high dose. As there were no associated abnormal histological findings and no functional deficit, the effect was considered not to be adverse. An increased incidence of extramedullary haematopoiesis in the livers of high-dose F_1 males only (8/30 versus 2/30 in controls) was also considered not to be adverse, as it is a common finding in rats and it was not seen in females or in F_0 males.

Litter sizes, pup viability, survival, lactation indices, body weights and body weight gains were not significantly different between the control and treated groups. No treatment-related clinical signs were seen in pups. Gross external and internal examination of the pups revealed no effect of treatment.

The NOAEL for parental, offspring and reproductive toxicity was 10 000 ppm (equal to 622 mg/kg bw per day), the highest dose tested (Beyrouty, 1996b).

(b) Developmental toxicity

Rats

In a developmental toxicity study, performed according to OECD Test Guideline No. 414, groups of 20 mated female Wistar rats were treated orally, by gavage, with NAG (purity unknown, supplied as 77.5% solution) in distilled water at a limit dose level of 1000 mg/kg bw per day or with vehicle only from day 7 through day 16 of gestation (day 0 = day of observation of copulatory plug). All rats were observed for clinical signs pretest and daily during the study. Body weight was recorded at days 0, 7, 14, 17 and 21 of pregnancy. The dams were killed and delivered by caesarean section on day 21 of pregnancy and examined macroscopically for abnormalities. The numbers of live and dead fetuses and the conceptuses undergoing resorption were determined. Body weight, crown–rump lengths, sex ratios of the fetuses and placental weights were recorded, and external, visceral and skeletal examinations of the fetuses were performed. In a range-finding study conducted beforehand, NAG had been administered orally to two groups of three gravid Wistar rats at dose levels of 111.1 and 1000 mg/kg bw per day from day 7 to day 16 of pregnancy. On day 21 of gestation, the dams were killed. Both doses were tolerated without untoward effects; therefore, a limit test at 1000 mg/kg bw per day was conducted. Statements of adherence to QA and GLP were included.

All rats survived until the termination of the study. There were no signs of disturbance of behaviour or general health, and NAG had no effect on body weight gain. Feed consumption of the dams treated with NAG was slightly lower during the treatment period than that of the controls. However, as feed consumption remained within the range of historical control values and body weight gain was not adversely affected, no toxicological significance was attached to this finding. No adverse

effects were observed at examination after caesarean section. At necropsy and body cross-sectional examination of fetuses, it was found that one eyelid of a fetus in the substance group was half open. Blood was present in the pericardium or abdominal cavity of some fetuses in the substance group and isolated fetuses in the control group. Some of the fetuses in both the substance and control groups exhibited unilateral or bilateral distension of the renal pelvis, accompanied frequently by distension of the corresponding ureter(s). Distension of the ureter was found in one fetus from the treatment group. The skeletons of the treatment group fetuses were at much the same stage of development as those of the control fetuses. The degree of ossification corresponded to day 21 of gravidity. One NAG-exposed fetus had only six vertebral arches on the right side in the cervical region of the vertebral column, and one control fetus showed thickened ribs on both sides. Otherwise, no adverse effects were seen. The terminal necropsy revealed no macroscopically visible abnormalities in the internal organs of any of the dams. The heart, liver, kidney and spleen weights of the NAG-treated dams were comparable to those of control animals.

NAG was not teratogenic to the rat when dosed at 1000 mg/kg bw per day. The NOAEL for maternal and developmental toxicity was 1000 mg/kg bw per day, the only dose tested (Horstmann & Baeder, 1992; Baeder, Albrecht & Mayer, 1993).

Rabbits

An oral developmental toxicity study was performed according to OECD Test Guideline No. 414 with Himalayan rabbits (15 per group) exposed to NAG (purity 92.4%, supplied as 77.5% w/w aqueous solution) at 0, 64, 160 or 400 mg/kg bw per day by oral gavage once daily. Rabbits were mated with sexually mature males at a ratio of 1:1 and again 6 hours later. Dams were administered once daily with NAG from GD 6 to GD 18 and sacrificed on day 29 of pregnancy. All rabbits were observed daily for clinical signs. Body weights and feed consumption were recorded on days 0, 6, 13, 19 and 29 of gestation. At necropsy, dams were examined for macroscopically visible changes. The uterus was examined to determine the reproductive status (number of live and dead fetuses and the number of conceptuses undergoing resorption). Live fetuses were removed and checked for viability during 24 hours. Body weights, crown–rump lengths, sex ratios of the fetuses and placental weights were determined, and external, visceral and skeletal examinations of the fetuses were performed. Statements of adherence to QA and GLP were included.

None of the females died. Body weight development of the dams remained unaffected.

Reduced water consumption and pultaceous faeces were observed sporadically in two animals and one animal of the 400 mg/kg bw per day group, respectively. Body weight gain was not impaired by administration of the test substance. Feed consumption was slightly to moderately decreased in the animals of the intermediate-dose group (160 mg/kg bw per day) and high-dose group (400 mg/kg bw per day) during the treatment period (Table 35).

Table 35. Mean feed consumption in a teratogenicity study in rabbits exposed to NAG

Days of gestation	Mean feed consumption (± standard deviation) (g/100 g bw)			
	0 mg/kg bw per day	64 mg/kg bw per day	160 mg/kg bw per day	400 mg/kg bw per day
0–6	4.21 (± 0.45)	4.23 (± 0.60)	4.16 (± 0.50)	4.26 (± 0.27)
6–13	3.69 (± 0.81)	3.17 (± 0.82)	2.79* (± 0.63)	2.20* (± 0.59)
13–19	3.81 (± 0.89)	3.41 (± 0.83)	2.97* (± 0.65)	2.67* (± 1.26)
19–29	4.03 (± 0.56)	3.90 (± 0.67)	3.96 (± 0.48)	4.34 (± 0.90)

From Baeder & Hofmann (1994)

* $P < 0.05$ (according to a non-parametric linear model)

No compound-related changes were observed at necropsy. All animals became pregnant. One female in the low-dose group had an abortion, and one in the intermediate-dose group had an early intrauterine death. Dead and stunted fetuses were observed in one animal of the low-dose group and in two animals of the intermediate- and high-dose groups, respectively. As these incidences were within the normal range of the rabbit strain used and there was no clear dose–response relationship, it was considered that these observations were not due to administration of the compound. Morphological examination of the fetuses revealed a statistically significant increase in the incidence of supernumerary thoracic ribs at 160 and 400 mg/kg bw per day. The incidence was within the normal range in the intermediate-dose group and slightly above the upper limit of the normal range in the high-dose group (Table 36).

Table 36. Intergroup comparison of extra thoracic rib incidence (by fetus/litter) in a teratogenicity study in rabbits exposed to NAG

	Incidence of extra rib (number/number examined (%))			
	0 mg/kg bw per day	64 mg/kg bw per day	160 mg/kg bw per day	400 mg/kg bw per day
Fetuses	2/90 (2.2%)	0/82	8/73* (11%)	11/90** (12%)
Litters	2/15 (13%)	0/14	7/14* (50%)	5/15 (33%)

From Baeder & Hofmann (1994)
* $P < 0.05$; ** $P < 0.01$ (according to the Fisher exact test, one-sided)

Administration of NAG at dose levels of 160 and 400 mg/kg bw per day from day 6 to day 18 of pregnancy caused slight to moderate decreases in feed consumption of the dams. The fetuses in these groups showed statistically significant increases in the incidence of extra thoracic ribs. The NOAEL for maternal toxicity was 64 mg/kg bw per day, based on reduced feed consumption at 160 mg/kg bw per day. The NOAEL for developmental toxicity was 64 mg/kg bw per day, based on an increased incidence of extra thoracic ribs at 160 mg/kg bw per day (Baeder & Hofmann, 1994; Hofmann, Baeder & Mayer, 1995; Ernst & Leist, 1999b).

5.6 Special studies

(a) Neurotoxicity

Rats

An acute oral (dose range–finding) neurotoxicity study was performed in accordance with USEPA Guideline OPPTS 870.6200 (1998) in Wistar (SPF) rats treated with 0, 100, 1000 or 2000 mg/kg bw (purity 33.8%, doses refer to amount of active substance using a correction factor of 2.96). Rats (10 of each sex per group) were given a single dose by oral gavage at approximately 9 weeks of age. Mortality and clinical signs were observed hourly up to 10 hours after dosing and twice daily during test days 2–15. Feed consumption and body weights were recorded periodically pretest and during the observation period. All animals were killed on test day 15, necropsied and examined post mortem. A functional observational battery was performed, and locomotor activity, body temperature, landing foot splay and grip strength were measured pretest and 1, 7 and 14 days after application. Statements of adherence to QA and GLP were included.

No deaths occurred during the study. No treatment-related findings were observed in the functional observational battery or on locomotor activity, grip strength, body temperature, rearing or landing foot splay. Diarrhoea, ruffled fur and sedation were observed in almost all animals treated with 2000 mg/kg bw on day 1. These findings had fully reversed by day 2. No clinical signs were observed in the low- or mid-dose groups. Feed consumption and body weight gain were unaffected in the test groups when compared with the control animals. No macroscopic findings were observed at necropsy.

The NOAEL was 1000 mg/kg bw, based on clinical signs (diarrhoea, ruffled fur and sedation) observed at 2000 mg/kg bw (Hamann, 1999c).

An acute oral neurotoxicity study, including a water maze test to assess learning, memory and relearning, was performed on groups of Wistar (SPF) rats (10 of each sex per group) treated by gavage with NAG (purity 33.8%, doses refer to amount of active substance using a correction factor of 2.96) at a single dose of 0, 100, 1000 or 2000 mg/kg bw. All animals were tested pretreatment and again 1, 7 and 14 days after dosing. The study was not performed in accordance with any specific guideline. Histological examinations were performed on brain, spinal cord, sciatic nerve and tibial nerve from all animals. The general observations were performed as described above (Hamann, 1999c). Statements of adherence to QA and GLP were included.

No treatment-related findings were observed in any of the animals during the learning, memory and relearning phases of the water maze test. No treatment-related macroscopic or microscopic findings were observed in any of the organs or tissues examined. The single oral administration of NAG to rats at doses of 100, 1000 and 2000 mg/kg bw did not result in any deaths. Treatment-related findings were restricted to clinical signs at 2000 mg/kg bw and comprised sedation, ruffled fur and diarrhoea on day 1.

The NOAEL was 1000 mg/kg bw, based on clinical signs (diarrhoea, ruffled fur and sedation) observed at 2000 mg/kg bw (Hamann, 1999d).

Groups of 10 male and 10 female Wistar rats were given NAG (purity 33.8%) at a dietary level of 0, 20, 200 or 2000 ppm (equal to 0, 1.6, 16 and 159 mg/kg bw per day for males and 0, 1.8, 18 and 179 mg/kg bw per day for females, respectively) for up to 38 days. An additional five rats of each sex were used at each dose level for determining glutamine synthetase activity in the liver, kidney and brain at the end of the treatment period. The animals were observed daily for clinical signs, whereas body weight and feed consumption were recorded weekly. A functional observational battery, locomotor activity, body temperature, landing foot splay distance and grip strength were measured pretest and after 2 and 4 weeks of treatment. A water maze test was performed pretest and in week 5. A single dose of midazolam (2 mg/kg bw) given intraperitoneally before or 10 minutes after the water maze test was used as a positive control. At termination, all the animals were killed and examined macroscopically. The brain, heart, liver and kidneys were weighed, and histopathological examination was performed on the brain, spinal cord, and sciatic and tibial nerves of all the control and high-dose animals. Statements of adherence to QA and GLP were included.

No treatment-related mortality or clinical signs were observed. Body weight gain, feed consumption, functional observational battery parameters, locomotor activity, rearing, grip strength, body temperature, landing foot splay, or learning and memory tests (water maze) were unaffected by treatment with NAG.

In animals receiving NAG, a statistically significant inhibition of glutamine synthetase activity was observed in the liver of males at 200 and 2000 ppm and in females at 2000 ppm (Table 37). Glutamine synthetase activity in kidneys was decreased by NAG in high-dose males. The slight reduction in glutamine synthetase activity in males at 20 ppm was considered incidental, in view of the lack of dose dependency. No inhibition of glutamine synthetase activity was observed in the brain tissue of animals receiving NAG. In the absence of histopathological findings, the relatively small reductions in glutamine synthetase activity in liver and kidneys are considered not adverse.

No treatment-related macroscopic or microscopic abnormalities were seen in any of the animals.

The NOAEL for NAG was 2000 ppm (equal to 159 mg/kg bw per day), the highest dose tested (Hamann et al., 2000).

Table 37. Mean glutamine synthetase activity following 38 days of treatment with NAG in rats

Organ	% of control activity					
	Males			Females		
	20 ppm	200 ppm	2000 ppm	20 ppm	200 ppm	2000 ppm
Liver	100	79*	71**	104	95	79*
Kidney	88*	90	73**	107	97	101
Brain	98	100	95	97	95	98

From Hamann et al. (2000)
* $P < 0.05$; ** $P < 0.01$ (according to the parametric method of Dunnett)

The effect of subchronic treatment with NAG on the activity of glutamine synthetase was investigated in liver, kidneys and brain. NAG (supplied as an aqueous technical concentrate; concentration 33.8% w/w) was administered in the diet to groups of 10 male Wistar rats for 6, 13, 20 or 90 days at a dose of 1000 or 10 000 ppm. Additional groups of 10 male Wistar rats received NAG at a dose of 1000 or 10 000 ppm for 91 days followed by a 30-day recovery period. Control groups of 10 male Wistar rats received the untreated diet for the same period of time. Achieved nominal intakes of NAG were 63.2 and 658 mg/kg bw per day at 1000 and 10 000 ppm, respectively. Mortality and clinical signs were recorded daily, and feed consumption and body weights were measured weekly. All animals were necropsied at scheduled sacrifice. Organ weights (brain, liver and kidney) were measured, and all macroscopic findings were recorded. Samples of liver, kidney and brain were collected from all animals and processed for measurement of glutamine synthetase activity. Statements of adherence to QA and GLP were included.

No treatment-related effects were noted for clinical signs, feed consumption or body weight.

NAG induced a significant inhibition of glutamine synthetase activity in the liver and kidney (see Table 38). No toxicologically relevant effect of NAG on glutamine synthetase activity in the brain was observed. No cumulative effect over time was observed. The effects were (almost) completely reversed after a 30-day recovery period.

Table 38. Inhibition of glutamine synthetase activity in the rat by NAG

Tissue	Sacrifice after day:	% inhibition	
		1000 ppm	10 000 ppm
Liver	6	4	54**
	13	24**	60**
	20	26**	43**
	90	42**	46**
	Recovery	17*	6
Brain	6	6	7*
	13	0	0
	20	2	4
	90	1	2
	Recovery	3	3
Kidney	6	39**	46**
	13	41**	46**
	20	32**	45*
	90	45**	47**
	Recovery	5	13

From Schmid et al. (1999)
* $P < 0.05$; ** $P < 0.01$ (according to the Dunnett test based on pooled variance)

There were no treatment-related macroscopic findings or effects on liver and brain weights. Necropsy at 6, 13 or 20 days showed an increase in absolute and relative kidney weights in rats treated with NAG (up to 22%) that was fully reversible after 30 days of recovery.

The NOAEL for NAG was 1000 ppm (equal to 63.2 mg/kg bw per day), based on a greater than 50% reduction in glutamine synthetase activity in the liver at 10 000 ppm (equal to 658 mg/kg bw per day) (Schmid et al., 1999).

The effect of NAG (concentration 33.8% w/w) at doses up to 10 000 µg/ml on the in vitro activity of glutamine synthetase was investigated in samples of liver, kidney and brain (neocortex, medulla oblongata and hypothalamic region) of Wistar rats. Statements of adherence to QA and GLP were included.

NAG induced no significant inhibition up to 1000 µg/ml. At 5000 and 10 000 µg/ml, a minimal level of inhibition was observed, reaching 9% and 15%, respectively, which can be attributed to the presence of glufosinate-ammonium as an impurity and the metabolic deacetylation of NAG to form glufosinate-ammonium. NAG induced no inhibition of glutamine synthetase in kidney over the whole range of concentrations tested.

In the brain (neocortex, medulla and hypothalamus), NAG did not induce any inhibition up to 1000 µg/ml, whereas at 5000 and 10 000 µg/ml, only a minimal inhibition was observed (up to 7%). This can be attributed to the presence of glufosinate-ammonium as an impurity and the metabolic deacetylation of NAG to form glufosinate-ammonium (Luetkemeier, 1999).

6. Observations in humans

No information is available.

C. 3-METHYLPHOSPHINICO-PROPIONIC ACID (MPP)

7. Biochemical aspects

7.1 Absorption, distribution and excretion

[^{14}C]MPP (radiochemical purity 98%) was administered either intravenously or orally by gavage at a single dose of 20 mg/kg bw to groups of five female SPF Wistar rats. The vehicle was an aqueous sodium solution. Urine and faeces were collected on a daily basis for 96 hours and radioassayed by LSC.

Total recovery of radioactivity was 96% and 93% for the oral and intravenous groups, respectively. After oral administration, 92% and 4% of the radiolabel was excreted in urine plus cage wash and faeces, respectively. Over the first 24 hours, 83% and 3% were excreted in urine and faeces, respectively. After intravenous administration, 93% and 0.6% were excreted in urine and faeces, respectively. After intravenous administration, 87% of the dose was excreted in urine over the first 24 hours (Kellner & Eckert, 1984).

7.2 Biotransformation

No information was available.

8. Toxicological studies

8.1 Acute toxicity

(a) Lethal doses

The results of studies of acute toxicity with MPP are summarized in Table 39. Treatment-related mortality and signs of toxicity were observed during the acute oral studies.

Table 39. Results of studies of acute toxicity with MPP

Species	Strain	Sex	Route	Vehicle	Purity (%)	LD_{50} (mg/kg bw)	Reference
Mouse	NMRI	M/F	Oral	2% potato starch in deionized water	> 99.8	3050 (M) 3070 (F)	Diehl & Leist (1988a)[a]
Rat	Wistar	M/F	Oral	Water	> 99	2840 (M) 1900 (F)	Rupprich & Weigand (1984)[b]

F, female; LD_{50}, median lethal dose; M, male

[a] The following clinical signs were observed: reduced spontaneous activity, squatting position, contracted flanks, piloerection, high-legged gait, narrowed palpebral fissures, irregular breathing, reduced placing reflex, ataxic gait, prone or lateral position, gasping, increased respiratory rate, straddling of hind legs, hypersensitivity to touch, crawling locomotion and jerky breathing. Deaths occurred on the 1st day of treatment and up to the 10th day after treatment. Necropsy of animals that died revealed abnormalities in the stomach and intestine (inflated or fluid filled) and various pale-coloured organs. Dissection of the animals killed at the end of the study revealed no macroscopically visible abnormalities.

[b] The following clinical signs were observed: squatting position, contracted flanks, abdominal position, uncoordinated gait, piloerection, quiet behaviour, drowsiness, narrowed eye openings and jerky respiration. Some animals also showed skin pallor, noisy respiration and widening of the palpebral fissures. These clinical signs were more pronounced at the higher dose levels during the observation period. Lethally intoxicated animals died between 23 minutes and 3 days after administration. By day 7 after treatment, practically all of the animals were free of clinical signs. Macroscopic examination of the animals found dead showed petechial haemorrhages in the gastric mucosa, slight quantities of a blood-coloured mass in the stomach, stomach taut with brownish-yellow fluid and feed, a blackish-brown mass in the small intestine, clear to yellowish fluid in the small intestine, mucosa of small intestine whitish in colour, adrenals dark in colour, liver light-coloured in places, lungs congested with blood and lungs greenish-brown in colour. The animals killed at the end of the observation period were free of macroscopically visible changes.

(b) Dermal sensitization

In a dermal sensitization study using the Magnusson and Kligman maximization test, performed in accordance with OECD Test Guideline 406, MPP (purity > 99.8%) was tested in 20 female Pirbright-White guinea-pigs. A preliminary study established 1% and 50% test substance concentrations in saline as suitable for the intradermal induction and dermal induction and challenge phases, respectively. The control group consisted of 10 animals. In the first induction phase, the animals were subjected to two intradermal injections of 50% Freund's Complete Adjuvant, 1% MPP in isotonic saline and 1% MPP in 50% Freund's Complete Adjuvant. Seven days later, the same area of skin was treated by topical application of 0.5 ml of a 50% solution of MPP in saline and the test site covered with an occlusive dressing for 48 hours. The same induction procedure was carried out on control groups with vehicles only. On day 22, all animals were challenged by a 24-hour occluded topical application of 50% MPP in saline. The test sites were assessed 24 and 48 hours after removal of the occlusive bandages. Benzocaine was used as a positive control. Statements of adherence to QA and GLP were included.

The intradermal injections with Freund's Complete Adjuvant (with and without test substance) caused well-defined erythema and slight oedema. After the dermal challenge treatment on day 22, two treated animals showed very slight erythema. Under the conditions of this study, MPP was not a skin sensitizer (Diehl & Leist, 1988b).

8.2 *Short-term studies of toxicity*

Mice

Groups of 10 male and 10 female NMRI mice received MPP for 13 weeks at a dietary concentration of 0, 320, 1600, 3200 or 8000 ppm (equal to 0, 55, 264, 522 and 1288 mg/kg bw per day for males and 0, 57, 279, 590 and 1540 mg/kg bw per day for females, respectively). Mortality and clinical signs were checked daily. Body weight and feed and water consumption were recorded weekly. Haematological examinations, clinical chemistry and urine analysis were performed at the end of the study. At termination, all animals were examined macroscopically, and selected organs were weighed. All major organs and tissues were examined microscopically. Statements of adherence to QA and GLP were included.

No mortalities or clinical signs were observed. Body weight gain, feed and water consumption and haematological and urine analysis parameters were not affected by treatment. In males of the high-dose group, a 41% reduction in serum uric acid was observed. At the high dose, relative kidney weights were increased in males (12%) and females (9%). As no treatment-related histopathological changes were found, these changes in relative organ weight were not considered adverse.

The NOAEL was 8000 ppm (equal to 1288 mg/kg bw per day), the highest dose tested (Ebert & Leist, 1989).

Rats

In a dietary range-finding study, Wistar rats (five of each sex per group) were fed diets containing MPP (purity > 99%) for 4 weeks at a concentration of 0, 50, 500, 2500 or 5000 ppm (equal 0, 5.6, 57, 286 and 554 mg/kg bw per day for males and 0, 5.5, 55, 282 and 561 mg/kg bw per day for females, respectively). Rats were examined daily for clinical signs of toxicity. Body weight and feed consumption were measured twice weekly during the study. Water consumption was measured weekly. Haematology, serum biochemical determinations, glutamine synthetase activity in the liver and urine analyses were performed after 4 weeks. At the end of the treatment period, the rats were necropsied, and selected organs were weighed. A wide range of organs of control and high-dose animals was examined microscopically. Statements of adherence to QA and GLP were included.

No deaths or clinical signs of systemic toxicity were observed. No toxicologically relevant effects on body weight gain, feed and water consumption, haematology, clinical chemistry, urine analysis or pathology were observed. Occasionally observed increases in haemoglobin, uric acid and triglyceride levels were within normal biological variation. In the high-dose females, there was a 9% increase in relative liver weight; however, it was not accompanied by histopathological changes, and it was therefore considered not to be toxicologically adverse (Ebert, Leist & Mayer, 1986).

In a 90-day dietary study, groups of 10 male and 10 female Wistar rats received MPP (purity 99.6%) at a concentration of 0, 400, 1600 or 6400 ppm (equal to 0, 34, 127 and 546 mg/kg bw per day for males and 0, 36, 141 and 570 mg/kg bw per day for females, respectively). Additional groups of 10 animals of each sex were maintained for a 4-week post-dosing recovery period. Animals were checked daily for mortality and clinical signs of toxicity. A detailed clinical examination, including a neurological assessment, was performed weekly. Body weights and feed and water consumption were measured weekly. Ophthalmological examinations were carried out pretest and at week 11. Haematology, clinical chemistry and urine analysis were performed at the end of treatment and at the end of the recovery period. At termination, the animals were examined macroscopically, and the major organs were weighed. A wide range of organs and tissues was examined histopathologically. Statements of adherence to QA and GLP were included.

No mortality occurred. No treatment-related effects on body weight gain, feed and water consumption or ophthalmoscopy were observed. Slight, but statistically significant, increases in

reticulocyte numbers in mid- and high-dose males and in lactate dehydrogenase levels in mid-dose females were found. Also, significant increases or decreases in α-, β- and γ-globulin were occasionally observed. However, as the effects were generally not dose dependent and were observed in one sex only, these changes were considered not to be toxicologically relevant. At the high dose, mild increases (up to 12%) in absolute or relative liver weights (males only) and kidney weights (both sexes) were observed at termination or in the recovery animals. In the absence of histopathological or clinical chemistry evidence for an effect of treatment on these organs, the organ weight changes were not considered adverse.

The NOAEL was 6400 ppm (equal to 546 mg/kg bw per day), the highest dose tested (Ebert & Mayer, 1987).

Dogs

In a 28-day oral range-finding study, groups of three male and three female Beagle dogs received MPP (purity 99.2%) in the diet at 0, 100, 320 or 1000 ppm (equal to 0, 7, 22 and 70 mg/kg bw per day for males and 0, 6, 19 and 58 mg/kg bw per day for females, respectively). After treatment, one animal of each sex per dose was kept for a 4-week recovery period. The dogs were observed daily for mortality and clinical signs. Body weight and feed and water consumption were measured weekly. The neurological status, ophthalmoscopy, a hearing test, haematology, clinical chemistry (including measurement of acetylcholinesterase activity in plasma, erythrocytes and brain) and urine analysis were assessed before dosing started and before termination of the study. At the end of the recovery period, haematology, clinical chemistry and urine analysis were also performed. At termination, all animals were necropsied, and major organs were weighed. A wide range of organs and tissues was examined histologically. Glutamine synthetase activity in the liver, kidney and brain was assessed. Statements of adherence to QA and GLP were included.

No mortality occurred. None of the dogs showed treatment-related effects on any of the parameters examined (Brunk & Mayer, 1987).

In a 90-day dietary study, MPP was administered to groups of Beagle dogs at a level of 0, 100, 400 or 1600 ppm (equal to 0, 7, 29 and 115 mg/kg bw per day for males and 0, 6, 26 and 103 mg/kg bw per day for females, respectively). All groups consisted of six animals of each sex, except for the low-dose group, which consisted of four dogs of each sex. Two males and two females each for the control, mid- and high-dose groups were kept under observation for a recovery period of about 4 weeks. Viability, clinical signs and feed consumption were assessed daily. Body weights were measured weekly. Neurological and hearing tests, ophthalmology, haematology, clinical chemistry and urine analysis were performed pretreatment, after approximately 6 weeks, before termination of the main phase of the study and towards the end of the recovery period. The hepatic and renal functions were tested with BSP and PSP at the same time points. At necropsy, organs were examined macroscopically and weighed, and selected organs and tissues were examined histopathologically. Glutamine synthetase activity in liver, kidney and brain was determined in all dogs at the time of sacrifice. Statements of adherence to QA and GLP were included.

No mortalities or signs of toxicity were noted. There was no effect on feed consumption or body weight. Neurological, ophthalmological and hearing tests and haematology and urine analysis revealed no effect of treatment. A number of clinical chemistry parameters showed small, but statistically significant, changes. However, often these findings were not dependent on dose, were not found consistently over the time points and were found in one sex only. These findings were considered to be incidental and not related to treatment. Glutamine synthetase activity and liver and kidney function were not affected by treatment. Macroscopic, histopathological and organ weight examination revealed no treatment-related effects.

The NOAEL was 1600 ppm (equal to 103 mg/kg bw per day), the highest dose tested (Brunk, 1988).

8.3 Long-term studies of toxicity and carcinogenicity

No information was available.

8.4 Genotoxicity

MPP was tested for genotoxicity in a limited range of guideline-compliant assays. No evidence for genotoxicity was observed.

The results of the genotoxicity tests are summarized in Table 40.

Table 40. Overview of genotoxicity tests with MPP[a]

End-point	Test object	Concentration	Purity (%)	Results	Reference
In vitro					
Point mutations	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538; *Escherichia coli* WP2 *uvrA*	4–5000 µg/plate (±S9)	> 99	Negative	Jung & Weigand (1984)[b,c]
Forward mutation	*Schizosaccharomyces pombe* strain P1	313–10 000 µg/ml (±S9)	99.8	Negative	Nunziata & Haroz, (1989)[b,d]
Mitotic recombination	*Saccharomyces cerevisiae* D4	625–10 000 µg/ml (±S9)	95.3	Negative	Forster (1989)[b,e]
Chromosomal aberrations	Human lymphocytes	100–1520 µg/ml (±S9)	99.6	Negative	Heidemann (1988)[f]
Gene mutation	V79 Chinese hamster cells, HPRT test	100–1000 µg/ml (±S9)	99.6	Negative	Müller (1988)[g]
Unscheduled DNA synthesis	Human cell line A 549	1–2000 µg/ml	99.6	Negative	Kramer & Müller (1987)[h]
In vivo					
Chromosomal aberrations	Chinese hamster bone marrow	0, 100, 333 or 1000 mg/kg bw (gavage)	99.8	Negative	Voelkner & Müller (1988)[i]
Micronucleus formation	NMRI mouse bone marrow	0, 200, 600 or 2000 mg/kg bw (gavage)	96.9	Negative	Müller (1989e)[j]

DNA, deoxyribonucleic acid; S9, 9000 × *g* supernatant fraction of rat liver homogenate

[a] Positive and negative (solvent) controls were included in all studies.

[b] Statements of adherence to QA and GLP were included.

[c] Toxicity was observed at doses of 500 µg/plate and higher. A small increase in number of revertant colonies of TA1535 at 5000 µg/plate was attributed to cytotoxicity observed at this dose level.

[d] Batch Ka 417/II.

[e] Batch Ka 417/II. Test design resembles OECD Test Guideline No. 481.

[f] Batch H404. Performed in accordance with OECD Test Guideline No. 473.

[g] Batch H404. Performed in accordance with OECD Test Guideline No. 476.

[h] Batch H404. Test design resembles OECD Test Guideline No. 482.

[i] Batch Ka 417/II. Performed in accordance with OECD Test Guideline No. 475. In a range-finding test, all animals at 1500, 2000 and 4000 mg/kg bw expressed signs of toxicity, such as reduction of spontaneous activity, eyelid closure and apathy. Mortalities were also observed. At 1000 mg/kg bw, all animals survived despite exhibiting signs of toxicity.

Table 40 (continued)

[j] Batch KA 417/II. Study design resembles OECD Test Guideline No. 474. At 2000 mg/kg bw, one female died 24 hours after treatment, and another animal was killed because of an unusual oedema in the right anterior leg. These animals displayed reduced spontaneous activity, narrowed palpebral fissures, forward movement in crawling posture and piloerection. The animals that survived were all free of clinical signs of toxicity. The ratio of polychromatic erythrocytes to normocytes remained essentially unaffected by the test compound; thus, there is no clear evidence that MPP reached the bone marrow. However, as sublethal doses were used, it is likely that the substance reached the systemic circulation and the target tissue.

8.5 Reproductive and developmental toxicity

(a) Multigeneration studies

No information is available.

(b) Developmental toxicity

Rats

In a developmental toxicity study, groups of 20 mated female Wistar rats were treated by gavage with MPP (purity 99.6%) in distilled water at a dose level of 0, 100, 300 or 900 mg/kg bw per day from days 7 through 16 of gestation (day 1 = day on which sperm were detected in the vaginal smear). Clinical signs were recorded daily. Body weight and feed consumption were measured weekly. All females were killed on day 21 of gestation and examined macroscopically, and selected organs were weighed. The uterus was examined, and the numbers of live and dead fetuses, resorptions, corpora lutea and implantations were counted. Body weight, crown–rump lengths and sex ratios of the fetuses as well as placental weight and diameter of fetuses undergoing resorption were recorded. About half of the fetuses from each litter were selected for skeletal examinations, and the other half for cross-sectional visceral examinations. Statements of adherence to QA and GLP were included.

At 900 mg/kg bw per day, one female died on GD 11 after having shown signs of flabbiness, disequilibrium, piloerection and increased urinary excretion. At this dose, feed consumption (6.75 g/100 g bw versus 8.62 g/100 g in controls) and body weight gain (18.9 g versus 32.3 g in controls) were reduced during the 1st week of treatment, and 10 females displayed piloerection persisting for several days. Two dams in this dose group had a bloody secretion in the vagina on days 13 and 15 of pregnancy, respectively. At 100 and 300 mg/kg bw per day, no evidence of treatment-related clinical signs or effects on feed consumption or body weight gain was found. At termination, no treatment-related macroscopic changes were found in any of the dose groups. Absolute kidney weight was increased by 19% in the high-dose females.

Three dams of the high-dose group and one dam of the mid-dose group lost all their conceptuses. No effects of treatment on fetal body weights and body lengths, sex ratio, placental weight, or incidence of skeletal and visceral abnormalities were found. At the high dose, an increased number of fetuses with wavy and/or thickened ribs was observed (15 versus 6 in the control group). However, the incidence of this finding was within the historical control range, and, in the absence of other developmental effects, it is considered unlikely to be related to treatment.

The NOAEL for maternal toxicity was 300 mg/kg bw per day, on the basis of one mortality, clinical signs of toxicity, reduced body weight gain and reduced feed consumption observed at 900 mg/kg bw per day. The NOAEL for fetal toxicity was 300 mg/kg bw per day, on the basis of three dams with total litter loss at 900 mg/kg bw per day. There was no evidence that MPP had a teratogenic effect (Albrecht & Baeder, 1994a).

Rabbits

Groups of 15 mated Himalayan rabbits received, by gavage, MPP at 0, 50, 100 or 200 mg/kg bw per day from days 6 to 18 of gestation. The vehicle was distilled water. The dose levels were

based on a range-finding study. Animals were examined daily for clinical signs and mortality. Body weight and feed consumption were recorded on GDs 0, 6, 13, 19 and 29. At GD 29, the dams were killed and examined macroscopically for abnormalities, and selected organs were weighed. The uterus was examined, and the numbers of live and dead fetuses and resorptions were determined. The live fetuses were removed and checked for viability for 24 hours. Body weights, crown–rump lengths, sex ratios of the fetuses and placental weights were recorded, and external, visceral and skeletal examinations of the fetuses were performed. Statements of adherence to QA and GLP were included.

Five dams from the high-dose group and one from the mid-dose group died during the study. In addition, four dams from the high-dose group and one from the 100 mg/kg bw per day group aborted and were killed. In these animals, signs of intoxication, such as disequilibrium, were observed, accompanied by reduced feed and water consumption and body weight reductions. In the surviving animals, body weight gains, feed consumption and the results of the macroscopic examination showed no effect of treatment. All surviving females in all groups carried live fetuses to full term. The number of corpora lutea, number of implantations and number of live fetuses in the treated dams that carried live fetuses to full term were not different from those for control dams. In the mid-dose dam that died and one high-dose dam that died, the conceptuses corresponded to the stage of gravidity at the time of death. Two of the five dams of the 200 mg/kg bw per day group that died had severely stunted fetuses, whereas two others had dead conceptuses undergoing resorption. In the high-dose dams killed due to suspected abortions, three dams had only 5–8 conceptuses, which perished at an early stage of gravidity and were undergoing resorption, whereas one dam had 9 conceptuses at the normal stage of development. In the 100 mg/kg bw per day group, the dam killed on day 22 due to suspected abortion had seven conceptuses undergoing resorption and a single live fetus that was normally developed. In all treatment groups, the dams that survived until termination showed no impairment of the intrauterine development of the conceptuses, the fetuses were normally developed and there was no increase in the number of dead conceptuses, except for one high-dose dam that had only one live fetus together with nine resorptions.

The live fetuses delivered in the treatment groups were normally developed. The body weights, body lengths, sex ratio, placental weight or placental gross appearance did not differ from controls. The survival rate of the fetuses 24 hours after delivery was high in all treated groups and showed no difference compared with the control group. There was no treatment-related increase in the incidence of external, skeletal and visceral malformations and variations. In the 50 mg/kg bw per day group, the number of fetuses with fewer than 13 ossified caudal vertebrae was increased. As a delay of skeletal ossification was not observed in the two higher-dose groups, this finding was considered not treatment related.

The NOAEL for maternal toxicity was 50 mg/kg bw per day, on the basis of one mortality, one abortion, clinical signs of toxicity, reduced body weight gain and reduced feed consumption observed at 100 mg/kg bw per day. The NOAEL for fetal toxicity was 50 mg/kg bw per day, on the basis of one dam with seven conceptuses undergoing resorption at 100 mg/kg bw per day. There was no evidence that MPP had a teratogenic effect (Albrecht & Baeder, 1994b).

8.6 Special studies

The capacity of MPP (purity > 99%) to inhibit glutamine synthetase activity was evaluated in addition to the other toxicology parameters. Groups of five male and five female Wistar rats received MPP in the diet at 0, 50, 500, 2500 or 5000 ppm (equal to 0, 5.6, 57, 286 and 554 mg/kg bw per day for males and 0, 5.5, 55, 282 and 561 mg/kg bw per day for females, respectively) for 28 days. The effect of MPP on glutamine synthetase activity in the liver was examined in the control and highest-dose groups. The effects on other toxicological parameters are described in the section on short-term toxicity (see above). Statements of adherence to QA and GLP were included.

No inhibition of glutamine synthetase activity in the liver was observed at 5000 ppm when compared with the control group. A slightly increased (approximately 10%) liver weight was

observed in females at 5000 ppm only. No change in kidney weight was observed (Ebert, Leist & Mayer, 1986).

9. Observations in humans

No information was available.

D. 2-METHYLPHOSPHINICO-ACETIC ACID (MPA)

10. Biochemical aspects

No information was available.

11. Toxicological studies

11.1 Acute toxicity

The results of studies of acute toxicity with MPA are summarized in Table 41. Treatment-related mortality and signs of toxicity were observed during the acute oral studies.

Table 41. Results of study of acute toxicity with MPA

Species	Strain	Sex	Route	Vehicle	Purity (%)	LD_{50} (mg/kg bw)	Reference
Rat	Sprague-Dawley	M/F	Oral	Deionized water	98.5	> 2000	Hammerl (1998)[a]

F, female; LD_{50}, median lethal dose; M, male

[a] Performed according to OECD Test Guideline No. 401. Batch 28688-92. Between 2 and 8 hours after administration, diarrhoea occurred in all treated rats. No other clinical signs were observed during the whole observation period.

11.2 Short-term studies of toxicity

Rats

In a 2-week dietary range-finding study, Wistar rats (six of each sex per group) were fed diets containing MPA (purity > 98.2%) at a concentration of 0, 2000, 5000 or 10 000 ppm (equal to 0, 243, 604 and 1231 mg/kg bw per day for males and 0, 248, 615 and 1128 mg/kg bw per day for females, respectively). Rats were examined daily for clinical signs of toxicity. Body weight and feed consumption were measured twice weekly. Haematology, serum biochemical determinations, glutamine synthetase activity in the liver and urine analyses were performed after 2 weeks. At the end of the treatment period, the rats were necropsied, and selected organs were weighed. The major organs of control and high-dose animals were examined microscopically.

No deaths or clinical signs of systemic toxicity were observed. No toxicologically relevant effects on body weight gain or feed consumption were observed. An increased body weight gain in mid- and high-dose females was attributed to a lower body weight of control females at the start of the test. Haematology, clinical chemistry and urine analysis showed no treatment-related effects. The few macroscopic or histological findings in control or treatment groups are considered to have occurred spontaneously and not to be treatment related (Syntin, 2001).

In a 90-day dietary study, groups of 10 male and 10 female Wistar rats received MPA (purity 98.3%) at a concentration of 0, 500, 2000 or 10 000 ppm (equal to 0, 34, 140 and 684 mg/kg bw per day for males and 0, 38, 156 and 772 mg/kg bw per day for females, respectively). Animals were checked daily for mortality and clinical signs of toxicity. A detailed clinical examination was performed weekly. Body weights and feed consumption were measured weekly. Ophthalmological

examinations was carried out pretest and at week 13. Haematology, clinical chemistry and urine analysis were performed at the end of treatment. The animals were tested in a functional observational battery during week 12. At termination, the animals were examined macroscopically, and the major organs were weighed. An extensive range of organs and tissues of the control and high-dose animals and all macroscopic lesions were examined histopathologically. Statements of adherence to QA and GLP were included.

No mortality or treatment-related clinical signs were found. Body weight gain, feed consumption and ophthalmoscopic, haematological, clinical chemistry and urine analysis parameters were not affected by treatment. The few differences that were occasionally noted were slight and within the historical control range. Postmortem examination revealed no treatment-related macroscopic, histological or organ weight changes.

The NOAEL was 10 000 ppm (equal to 684 mg/kg bw per day), the highest dose tested (Richard, 2001).

11.3 Long-term studies of toxicity and carcinogenicity

No information was available.

11.4 Genotoxicity

MPA was tested for genotoxicity in three guideline-compliant assays. No evidence for genotoxicity was observed in any test.

The results of the genotoxicity tests are summarized in Table 42.

Table 42. Overview of genotoxicity tests with MPA[a]

End-point	Test object	Concentration	Purity (%)	Results	Reference
In vitro					
Point mutations	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537; *Escherichia coli* WP2 *uvrA*	1.6–5000 μg/plate (±S9)	98.2	Negative	Ballantyne (2001b)[b,c]
Chromosomal aberrations	Human lymphocytes	745.9–1821 μg/ml (−S9) 1024–1821 μg/ml (+S9)	98.2	Negative	Lloyd (2001a)[b,d]
Gene mutation	Mouse lymphoma L5178Y *Tk* locus	56.25–1821 μg/ml (±S9)	98.2	Negative	Lloyd (2001b)[b,e]

DNA, deoxyribonucleic acid; S9, 9000 × *g* supernatant fraction of rat liver homogenate
[a] Positive and negative (solvent) controls were included in all studies.
[b] Statements of adherence to QA and GLP were included.
[c] Batch 28688-92. Performed in accordance with OECD Test Guideline No. 471.
[d] Batch 28688-92. Performed in accordance with OECD Test Guideline No. 476.
[e] Batch 28688-92. Performed in accordance with OECD Test Guideline No. 473.

11.5 Reproductive and developmental toxicity

No information was available.

12. Observations in humans

No information was available.

Comments

Glufosinate-ammonium

Biochemical aspects

After administration of single (2–500 mg/kg bw) or repeated oral doses (2 mg/kg bw), [^{14}C]glufosinate-ammonium was rapidly but incompletely absorbed (approximately 10%). Peak plasma concentrations were reached within 0.5–1 hour. The radiolabel was widely distributed, with highest concentrations in liver and kidneys. Radiolabel concentrations were low in the brain and fetus. The plasma half-life of the initial elimination phase was 4–5 hours. Excretion after single or repeated doses was rapid, with more than 90% excreted within 24 hours after administration of a low dose. Administration of higher doses resulted in slower absorption and excretion. In faeces, mainly glufosinate-ammonium and low concentrations (up to 10% of faecal radioactivity) of NAG were found, indicative of acetylation by microflora in the gut, as this metabolite is not found in urine. In urine, parent compound represented about 50% of the radioactivity, whereas MPB and MPP each represented 8–22% of the urinary radioactivity. Very low levels of MPA were found in urine. MPP represented 10–20% of residue found in liver. There were no marked sex differences in the kinetics and metabolism of glufosinate-ammonium.

Toxicological data

In 1999, the Meeting considered reports on the relevance of glutamine synthetase activity in the liver, kidney and brain of experimental animals and humans and concluded the following:

- A less than 50% inhibition of glutamine synthetase activity in rat liver was not associated with increased ammonia concentrations and thus was not considered to be adverse.
- Inhibition of kidney glutamine synthetase activity in the absence of pathological findings was not considered to be relevant to human risk assessment.
- Any statistically significant inhibition of glutamine synthetase activity in brain by more than 10% was considered a marker of potentially adverse effects on brain biochemistry and behaviour.

The present Meeting confirmed the conclusion of the 1999 JMPR, which is also supported by a recent published study on the essential role of glutamine synthetase in the implantation of mouse embryos.

The acute toxicity of glufosinate-ammonium is low in rats (LD_{50} > 1500 mg/kg bw; dermal LD_{50} > 2000 mg/kg bw; inhalation LC_{50} ≥ 1.26 mg/l). Glufosinate-ammonium is not irritating to the skin or eyes of rabbits and is not a skin sensitizer (Magnusson and Kligman test and Buehler test in guinea-pigs; local lymph node assay in mice).

In acute toxicity studies in mice, clinical signs of neurotoxicity were observed at 231 mg/kg bw (the lowest dose tested) and above. Mortality was observed at doses greater than or equal to 300 mg/kg bw.

In a single-dose toxicity study in dogs, clinical signs of neurotoxicity were observed at 200 mg/kg bw (the lowest dose tested), and mortality was observed at 400 mg/kg bw.

In three 13-week dietary studies in mice (two of them being range-finding studies), the overall NOAEL was 1280 ppm (equal to 278 mg/kg bw per day), based on clinical signs (ruffled fur, sedation, ventral recumbence or hunched posture, and emaciation) observed at 3500 ppm (equal to 561 mg/kg bw per day).

In a 28-day dietary range-finding and two 13-week dietary studies in rats, the overall NOAEL was 4000 ppm (equal to 263 mg/kg bw per day), based on neurological effects in both sexes and reduced body weight gain and feed consumption, reductions in erythrocyte count and low reticulocyte

ratios in males at 7500 ppm (equal to 521 mg/kg bw per day). Glutamine synthetase activity was not measured in these studies.

In a 28-day range-finding capsule study in dogs, the NOAEL was 1 mg/kg bw per day, based on reductions in glutamine synthetase activity in the central nervous system (8–53%), a slight increase in spontaneous motor activity that occurred within a few days after the start of treatment and reductions in body weight gain and feed consumption observed during the 1st week of treatment at 8 mg/kg bw per day. In a 90-day dietary study in dogs, the NOAEL was 64 ppm (equal to 2.0 mg/kg bw per day), based on a reduction in body weight gain and feed consumption in females at 256 ppm (equal to 7.8 mg/kg bw per day).

In a 1-year dietary study in dogs, mortality on days 10 and 14 and severe clinical signs, starting on day 9 of treatment, were observed after treatment with 375 ppm (equal to 10.6–16.0 mg/kg bw per day). The two deaths out of 16 animals at the high dose were caused by heart and circulatory failure attributed to marked myocardial necrosis in one dog and to severe necrotizing aspiration pneumonia in the other dog. After lowering the dose to 250 ppm (equal to 8.4 mg/kg bw per day), no adverse effects were observed. The NOAEL was 150 ppm (equal to 4.5 mg/kg bw per day). The study indicates that glufosinate-ammonium has a steep dose–response curve in dogs. In the absence of glutamine synthetase measurements in the 90-day and 1-year studies in dogs, an overall NOAEL of 1 mg/kg bw per day was established for these studies.

In a 2-year feeding study in mice, the NOAEL was 80 ppm (equal to 10.8 mg/kg bw per day), based on increased mortality and reduced body weight gain in males and changes in clinical chemistry parameters in both sexes at the next higher dose (equal to 23 mg/kg bw per day in males and 64 mg/kg bw per day in females). No effect on tumour incidence was observed.

In a 130-week feeding study in rats, the NOAEL was 140 ppm (equal to 7.6 mg/kg bw per day), based on effects on haematology, glutathione levels in liver and blood, and a reduction of brain glutamine synthetase activity at 500 ppm (equal to 26.7 mg/kg bw per day). In this study and a 2-year carcinogenicity study in rats, no effect on tumour incidence was found.

The Meeting concluded that glufosinate-ammonium is not carcinogenic in mice or rats.

Glufosinate-ammonium was tested for genotoxicity in an adequate range of studies of genotoxicity in vitro and in vivo. No evidence for genotoxicity was observed in any test.

The Meeting concluded that glufosinate-ammonium is unlikely to be genotoxic.

In view of the lack of genotoxicity and the absence of carcinogenicity in mice and rats, the Meeting concluded that glufosinate-ammonium is unlikely to pose a carcinogenic risk to humans.

A range-finding one-generation study and a two-generation study of reproductive toxicity in rats were available. The overall NOAEL for parental toxicity was 500 ppm (equal to 44 mg/kg bw per day), based on reduced feed consumption in males at 2500 ppm (equal to 206 mg/kg bw per day). At 2500 ppm and above, the dams delivered no pups. The overall NOAEL for offspring toxicity was 500 ppm (equal to 44 mg/kg bw per day), the highest dose at which dams produced a litter. The overall NOAEL for reproductive toxicity was 120 ppm (equal to 8.7 mg/kg bw per day), based on reduced litter sizes in all litters at 360 ppm (equal to 18 mg/kg bw per day). The Meeting considered the possibility that the increased preimplantation loss observed in the range-finding one-generation study of reproductive toxicity at 2500 ppm (equal to 207 mg/kg bw per day) might be caused by an inhibition of glutamine synthetase activity prior to implantation; a published mechanistic study in mice indicates that glutamine synthetase activity in preimplantation embryonic cells is essential for the blastocyst to complete implantation. The Meeting concluded that the preimplantation loss and early deaths in the reproductive toxicity studies might be caused by a single exposure to glufosinate-ammonium.

In three developmental toxicity studies in rats, the overall NOAEL for maternal toxicity was 10 mg/kg bw per day, based on clinical signs and abortions at 50 mg/kg bw per day. The overall

NOAEL for developmental toxicity was 10 mg/kg bw per day, based on intrauterine deaths at 50 mg/kg bw per day.

In a developmental toxicity study in rabbits, the NOAEL for maternal toxicity was 6.3 mg/kg bw per day, based on clinical signs, body weight loss and reduced feed consumption, an increased number of abortions and increased kidney weight at 20 mg/kg bw per day. The NOAEL for developmental toxicity was 6.3 mg/kg bw per day, based on an increased number of dead fetuses at 20 mg/kg bw per day.

The Meeting concluded that glufosinate-ammonium is not teratogenic in rats or rabbits.

In an acute gavage study of neurotoxicity in rats, the NOAEL was 100 mg/kg bw, based on clinical signs at 500 mg/kg bw.

In a dietary 38-day neurotoxicity study in rats and a 90-day dietary study investigating brain and liver glutamine synthetase inhibition in rats, the overall NOAEL was 100 ppm (equivalent to 6.2 mg/kg bw per day), based on a greater than 50% reduction in glutamine synthetase activity in the liver in males at 200 ppm (equal to 15 mg/kg bw per day).

In a dietary developmental neurotoxicity study in rats, the NOAEL for maternal toxicity was 1000 ppm (equal to 69 mg/kg bw per day), based on decreased body weight gain and feed consumption at 4500 ppm (equal to 292 mg/kg bw per day). The NOAEL for offspring toxicity was 200 ppm (equal to 14 mg/kg bw per day), based on reduced body weight gain during the preweaning period, effects on motor activity at postnatal days 17, 21 and 62, and hippocampal pathology in males at 1000 ppm (equal to 69 mg/kg bw per day).

Medical surveillance of plant production personnel did not find any effects related to the production of glufosinate-ammonium. Several human poisoning cases, sometimes leading to death, due to (suicidal) ingestion of glufosinate-ammonium have been reported in the literature. A variety of neurological symptoms have been described. It is not clear whether the toxicity was due to the active ingredient, to the surfactant contained in relatively high amounts in the formulation or to the combination of both.

Toxicological studies with the metabolites NAG, MPP and MPA, three metabolites that are found in plants, soil and livestock as well as in laboratory animals, were available. The toxicity of NAG, MPP and MPA is described separately below.

The Meeting concluded that the existing database on glufosinate-ammonium was adequate to characterize the potential hazards to fetuses, infants and children.

N-Acetyl-glufosinate (NAG)

Biochemical aspects

After administration of a single oral dose (3 mg/kg bw) of ^{14}C-labelled NAG to rats, NAG was rapidly but incompletely absorbed (approximately 5–10%). Peak plasma concentrations were reached within 1 hour. The highest residue levels were found in kidneys, followed by liver. Excretion after a single oral dose (3 mg/kg bw) was rapid, with approximately 95% of the absorbed dose excreted within 24 hours after administration. The absorbed NAG was predominantly excreted in urine. In faeces, mainly unchanged NAG was found, but about 10% was deacetylated to glufosinate by the intestinal microflora. In faeces, urine and tissues, minor amounts of MPP and MPA were found.

Toxicological data

The oral acute toxicity of NAG is low in rats and mice (LD_{50} > 2895 mg/kg bw). NAG is not a skin sensitizer (Magnusson and Kligman test in guinea-pigs).

In 4-week and 13-week dietary studies with NAG in mice, the overall NOAEL was 500 ppm (equal to 83 mg/kg bw per day), based on the inhibition of brain glutamine synthetase activity (11–13%) at 2000 ppm (equal to 233 mg/kg bw per day).

In a 4-week dietary range-finding study, two 13-week dietary studies and a 38-day dietary neurotoxicity study in rats, the overall NOAEL was 2000 ppm (equal to 159 mg/kg bw per day), based on statistically significant inhibition (11–12%) of liver glutamine synthetase activity at 10 000 ppm (equal to 658 mg/kg bw per day). Brain glutamine synthetase activity was reduced at 10 000 ppm (equal to 738 mg/kg bw per day). In the neurotoxicity study, no effects on glutamine synthetase and neurotoxicity parameters were observed at doses up to 2000 ppm (equal to 159 mg/kg bw per day), the highest dose tested.

In a 13-week dietary study in dogs, the NOAEL was 500 ppm (equal to 20 mg/kg bw per day), based on a reduction in brain glutamine synthetase activity (≥ 16%) at 2000 ppm (equal to 76 mg/kg bw per day).

In a 2-year dietary carcinogenicity study in mice, there were no toxicological findings and no increase in tumour incidence at the highest dose tested of 8000 ppm (equal to 1188 mg/kg bw per day). Glutamine synthetase activity was not measured.

In a 2-year dietary toxicity study in rats, the NOAEL was 2000 ppm (equal to 91 mg/kg bw per day), based on decreased body weight gain, increased incidence of soft faeces and increased incidences of polyarteritis nodosa in blood vessels and testes and urolithiasis at 20 000 ppm (equal to 998 mg/kg bw per day).

The Meeting concluded that NAG is not carcinogenic in mice or rats.

NAG was tested for genotoxicity in an adequate range of in vitro and in vivo studies. No evidence for genotoxicity was observed in any test.

The Meeting concluded that NAG is unlikely to be genotoxic.

In view of the lack of genotoxicity and the absence of carcinogenicity in mice and rats, the Meeting concluded that NAG is unlikely to pose a carcinogenic risk to humans.

In a range-finding one-generation study and a two-generation study of reproductive toxicity with NAG in rats, the NOAEL for parental, offspring and reproductive toxicity was 10 000 ppm (equal to 622 mg/kg bw per day), the highest dose tested.

In a developmental toxicity study in rats, the NOAEL for maternal and developmental toxicity was 1000 mg/kg bw per day, the only dose tested.

In a developmental toxicity study in rabbits, the NOAEL for maternal toxicity was 64 mg/kg bw per day, based on reduced feed consumption at 160 mg/kg bw per day. The NOAEL for developmental toxicity was 64 mg/kg bw per day, based on an increased incidence of extra thoracic ribs at 160 mg/kg bw per day.

The Meeting concluded that NAG is not teratogenic in rats or rabbits.

In two acute oral (gavage) studies of neurotoxicity in rats, the NOAEL was 1000 mg/kg bw, based on clinical signs (diarrhoea, ruffled fur and sedation) observed at 2000 mg/kg bw. No overt neurotoxicity was observed. Glutamine synthetase activity was not measured.

The Meeting concluded that the existing database on NAG was adequate to characterize the potential hazards to fetuses, infants and children.

3-Methylphosphinico-propionic acid (MPP)

Biochemical aspects

During the first 24 hours following administration of a single oral dose of ^{14}C-labelled MPP to rats, 83% and 3% of the radiolabel were excreted in urine and faeces, respectively.

Toxicological data

The acute oral toxicity of MPP is low in rats (oral LD_{50} = 1900 mg/kg bw). MPP is not a skin sensitizer (Magnusson and Kligman test in guinea-pigs).

In short-term dietary studies in mice (13 weeks, doses up to 8000 ppm, equal to 1288 mg/kg bw per day), rats (4 weeks and 13 weeks, doses up to 6400 ppm, equal to 546 mg/kg bw per day) and dogs (28 days and 90 days, doses up to 1600 ppm, equal to 103 mg/kg bw per day), no toxicity was observed. In the 4-week study in rats, glutamine synthetase activity in liver was not affected at doses up to 5000 ppm (equal to 554 mg/kg bw per day). In the two short-term studies in dogs, glutamine synthetase activity in liver, kidney and brain was not affected at doses up to 1600 ppm (equal to 103 mg/kg bw per day).

No long-term studies with MPP were available.

Glufosinate-ammonium was tested for genotoxicity in a limited range of studies in vitro. No evidence for genotoxicity was observed in any of these tests.

In a developmental toxicity study in rats, the NOAEL for maternal toxicity was 300 mg/kg bw per day, on the basis of one death out of 20 animals, clinical signs of toxicity and reduced body weight gain and feed consumption observed at 900 mg/kg bw per day. The NOAEL for fetal toxicity was 300 mg/kg bw per day, on the basis of 3 dams out of 20 with total litter loss at 900 mg/kg bw per day.

In a developmental toxicity study in rabbits, the NOAEL for maternal toxicity was 50 mg/kg bw per day, on the basis of one death out of 15 animals, one abortion, clinical signs of toxicity and reduced body weight gain and feed consumption observed at 100 mg/kg bw per day. The NOAEL for fetal toxicity was 50 mg/kg bw per day, on the basis of one dam with seven conceptuses undergoing resorption at 100 mg/kg bw per day.

The Meeting concluded that MPP is not teratogenic in rats or rabbits.

The Meeting concluded that the existing database on MPP was adequate to characterize the potential hazards to fetuses, infants and children.

2-Methylphosphinico-acetic acid (MPA)

The acute oral toxicity of MPA in rats was low (LD_{50} > 2000 mg/kg bw). In a 90-day dietary study in rats, the NOAEL was 10 000 ppm (equal to 684 mg/kg bw per day), the highest dose tested. Glutamine synthetase activity was not measured, but in view of the structural similarity between MPA and MPP, the Meeting considered it unlikely that MPA would inhibit this enzyme. MPA was not genotoxic in three genotoxicity tests in vitro.

Toxicological evaluation

The present Meeting compared the toxicity of NAG, MPP and MPA with that of glufosinate-ammonium and concluded that the toxicity of the metabolites was less than that of the parent compound. The Meeting established an ADI of 0–0.01 mg/kg bw for glufosinate-ammonium, on the basis of an overall NOAEL of 1 mg/kg bw per day, for reductions in glutamine synthetase activity in the brain of dogs. A safety factor of 100 was applied. This ADI also applies to its metabolites NAG, MPP and MPA. In view of the lower toxicity of NAG, MPP and MPA compared with glufosinate-ammonium, the Meeting noted that the application of the ADI to these metabolites is likely to be conservative. This ADI is considered to be adequately protective for any reproductive and developmental effects.

The Meeting established an acute reference dose (ARfD) for glufosinate-ammonium of 0.01 mg/kg bw, based on the NOAEL of 1 mg/kg bw per day in the 28-day capsule study in dogs for an increase in spontaneous motor activity that occurred within a few days after the start of treatment and reductions in body weight gain and feed consumption observed during the 1st week of treatment with 8 mg/kg bw per day and application of a safety factor of 100. This ARfD also applies to its

metabolites NAG, MPP and MPA. In view of the lower acute toxicity of NAG, MPP and MPA compared with glufosinate-ammonium, the Meeting noted that the application of the ARfD to these metabolites is likely to be conservative. This ARfD is considered to be adequately protective for any reproductive and developmental effects.

Levels relevant for risk assessment of glufosinate-ammonium

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year study of toxicity and carcinogenicity[a]	Toxicity	80 ppm, equal to 10.8 mg/kg bw per day	160 ppm, equal to 23 mg/kg bw per day
		Carcinogenicity	23 mg/kg bw per day[b]	—
Rat	Short-term studies of toxicity[c,d,e]	Toxicity	100 ppm, equal to 6.2 mg/kg bw per day	200 ppm, equal to 15 mg/kg bw per day
	Two-year study of toxicity and carcinogenicity[a]	Toxicity	140 ppm, equal to 7.6 mg/kg bw per day	500 ppm, equal to 26.7 mg/kg bw per day
		Carcinogenicity	500 ppm, equal to 26.7 mg/kg bw per day[b]	—
	One- and two-generation studies of reproductive toxicity[a,d]	Parental toxicity	500 ppm, equal to 44 mg/kg bw per day	2500 ppm, equal to 206 mg/kg bw per day
		Offspring toxicity	500 ppm, equal to 44 mg/kg bw per day[b]	—
		Reproductive toxicity	120 ppm, equal to 8.7 mg/kg bw per day	360 ppm, equal to 18 mg/kg bw per day
	Developmental toxicity study[c]	Maternal toxicity	10 mg/kg bw per day	50 mg/kg bw per day
		Embryo and fetal toxicity	10 mg/kg bw per day	50 mg/kg bw per day
	Developmental neurotoxicity study[c]	Maternal toxicity	1000 ppm, equal to 69 mg/kg bw per day	4500 ppm, equal to 292 mg/kg bw per day
		Embryo and fetal toxicity	200 ppm, equal to 14 mg/kg bw per day	1000 ppm, equal to 69 mg/kg bw per day
Rabbit	Developmental toxicity study[c]	Maternal toxicity	6.3 mg/kg bw per day	20 mg/kg bw per day
		Embryo and fetal toxicity	6.3 mg/kg bw per day	20 mg/kg bw per day
Dog	Short-term study of toxicity[f]	Toxicity	1 mg/kg bw per day	8 mg/kg bw per day

[a] Dietary administration.
[b] Highest dose tested.
[c] Gavage administration.
[d] Two or more studies combined.
[e] Based on inhibition of liver glutamine synthetase activity in a neurotoxicity study.
[f] Capsule administration.

Levels relevant for risk assessment of NAG

Species	Study	Effect	NOAEL	LOAEL
Mouse	Short-term studies of toxicity[a,b]	Toxicity	500 ppm, equal to 83 mg/kg bw per day	2000 ppm, equal to 233 mg/kg bw per day
	Two-year study of carcinogenicity[a]	Carcinogenicity	8000 ppm, equal to 1188 mg/kg bw per day[c]	—

Species	Study	Effect	NOAEL	LOAEL
Rat	Short-term studies of toxicity[a,b]	Toxicity	2000 ppm, equal to 159 mg/kg bw per day	10 000 ppm, equal to 658 mg/kg bw per day
	Two-year study of toxicity and carcinogenicity[a]	Toxicity	2000 ppm, equal to 91 mg/kg bw per day	20 000 ppm, equal to 998 mg/kg bw per day
		Carcinogenicity	20 000 ppm, equal to 998 mg/kg bw per day[c]	—
	One- and two-generation studies of reproductive toxicity[a,b]	Parental toxicity	10 000 ppm, equal to 622 mg/kg bw per day[c]	—
		Offspring toxicity	10 000 ppm, equal to 622 mg/kg bw per day[c]	—
		Reproductive toxicity	10 000 ppm, equal to 622 mg/kg bw per day[c]	—
	Developmental toxicity study[d]	Maternal toxicity	1000 mg/kg bw per day[c]	—
		Embryo and fetal toxicity	1000 mg/kg bw per day[e]	—
Rabbit	Developmental toxicity study[d]	Maternal toxicity	64 mg/kg bw per day	160 mg/kg bw per day
		Embryo and fetal toxicity	64 mg/kg bw per day	160 mg/kg bw per day
Dog	Short-term study of toxicity[a]	Toxicity	500 ppm, equal to 20 mg/kg bw per day	2000 ppm, equal to 76 mg/kg bw per day

[a] Dietary administration.
[b] Two or more studies combined.
[c] Highest dose tested.
[d] Gavage administration.
[e] Only dose tested.

Levels relevant for risk assessment of MPP

Species	Study	Effect	NOAEL	LOAEL
Mouse	Short-term study of toxicity[a]	Toxicity	8000 ppm, equal to 1288 mg/kg bw per day[b]	—
Rat	Short-term study of toxicity[a]	Toxicity	6400 ppm, equal to 546 mg/kg bw per day[b]	—
	Developmental toxicity study[c]	Maternal toxicity	300 mg/kg bw per day	900 mg/kg bw per day
		Embryo and fetal toxicity	300 mg/kg bw per day	900 mg/kg bw per day
Rabbit	Developmental toxicity study[c]	Maternal toxicity	50 mg/kg bw per day	100 mg/kg bw per day
		Embryo and fetal toxicity	50 mg/kg bw per day	100 mg/kg bw per day
Dog	Short-term study of toxicity[a]	Toxicity	1600 ppm, equal to 103 mg/kg bw per day[b]	—

[a] Dietary administration.
[b] Highest dose tested.
[c] Gavage administration.

Levels relevant for risk assessment of MPA

Species	Study	Effect	NOAEL	LOAEL
Rat	Short-term study of toxicity[a]	Toxicity	10 000 ppm, equal to 684 mg/kg bw per day[b]	—

[a] Dietary administration.
[b] Highest dose tested.

Estimate of acceptable daily intake for humans

0–0.01 mg/kg bw (ADI for glufosinate-ammonium, also applies to NAG, MPP and MPA)

Estimate of acute reference dose

0.01 mg/kg bw (ARfD for glufosinate-ammonium, also applies to NAG, MPP and MPA)

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of exposures in humans

Critical end-points for setting guidance values for exposure to glufosinate-ammonium and its metabolites NAG and MPP

	Glufosinate-ammonium	NAG	MPP
Absorption, distribution, excretion and metabolism in animals			
Rate and extent of absorption	Rapid, incomplete (~10%)	Rapid, incomplete (5–10%)	Rapid and complete (86%)
Distribution	Extensive; highest concentrations in liver and kidney	Extensive; highest concentrations in liver and kidney	No data
Potential for accumulation	Low	Low	Low
Rate and extent of excretion	> 90% within 24 h, primarily in faeces	> 95% within 24 h, primarily in faeces	86% within 24 h, primarily in urine
Metabolism in animals	Limited	Limited	No data
Toxicologically significant compounds in animals, plants and the environment	Glufosinate-ammonium, NAG, MPP, MPA	NAG, glufosinate-ammonium, MPP, MPA	MPP, MPA
Acute toxicity			
LD_{50}, oral, rat	> 1500 mg/kg bw	> 2895 mg/kg bw	1900 mg/kg bw
LD_{50}, dermal, rat	> 2000 mg/kg bw	No data	No data
LC_{50}, inhalation, rat	≥ 1.26 mg/l air	No data	No data
Rat, dermal irritation	Not an irritant	No data	No data
Rabbit, ocular irritation	Not an irritant	No data	No data
Dermal sensitization	Not a sensitizer (Magnusson & Kligman, Buehler, local lymph node assay)	Not a sensitizer (Magnusson & Kligman)	Not a sensitizer (Magnusson & Kligman)
Short-term studies of toxicity			
Target/critical effect	Brain (inhibition of glutamine synthetase) (dog)	Brain (inhibition of glutamine synthetase) (mouse, rat, dog)	None identified
Lowest relevant oral NOAEL	1 mg/kg bw per day (dog)	500 ppm, equal to 20 mg/kg bw per day (dog)	1600 ppm, equal to 103 mg/kg bw per day, highest dose tested (dog)
Lowest relevant dermal NOAEL	100 mg/kg bw per day (rat)	No data	No data

	Glufosinate-ammonium	NAG	MPP
Lowest relevant inhalation NOAEC	0.012 mg/l air	No data	No data
Long-term studies of toxicity and carcinogenicity			
Target/critical effect	Mortality, body weight gain, clinical chemistry (mouse) Haematology, brain glutamine synthetase (rats)	Body weight gain, clinical signs, polyarteritis nodosa in blood vessels and testes, urolithiasis (rat)	No data
Lowest relevant NOAEL	80 ppm, equal to 10.8 mg/kg bw per day (mouse) 140 ppm, equal to 7.6 mg/kg bw per day (rat)	2000 ppm, equal to 91 mg/kg bw per day (rat)	—
Carcinogenicity	Not carcinogenic (mouse, rat)	Not carcinogenic (rat)	—
Genotoxicity			
	Not genotoxic	Not genotoxic	Not genotoxic in a limited range of studies
Reproductive toxicity			
Reproduction target/critical effect	Reduced litter size (rat)	No reproductive target	No data
Lowest relevant parental NOAEL	500 ppm, equal to 44 mg/kg bw per day (rat)	10 000 ppm, equal to 622 mg/kg bw per day (rat)	—
Lowest relevant reproductive NOAEL	120 ppm, equal to 8.7 mg/kg bw per day (rat)	10 000 ppm, equal to 622 mg/kg bw per day (rat)	—
Lowest relevant offspring NOAEL	500 ppm, equal to 44 mg/kg bw per day (rat)	10 000 ppm, equal to 622 mg/kg bw per day (rat)	—
Developmental toxicity			
Developmental target	Intrauterine deaths (rat, rabbit)	Increased incidence of extra thoracic ribs (rabbit)	Intrauterine deaths (rat, rabbit)
Lowest relevant maternal NOAEL	10 mg/kg bw per day (rat) 6.3 mg/kg bw per day (rabbit)	64 mg/kg bw per day	50 mg/kg bw per day (rabbit)
Lowest relevant developmental NOAEL	10 mg/kg bw per day (rat) 6.3 mg/kg bw per day (rabbit)	64 mg/kg bw per day	50 mg/kg bw per day (rabbit)
Neurotoxicity			
Acute oral neurotoxicity NOAEL	1 mg/kg bw per day (dog); increased motor activity	Not neurotoxic (2000 mg/kg bw)	No data
Short-term neurotoxicity NOAEL	1 mg/kg bw per day (dog); inhibition of brain glutamine synthetase, increased motor activity	500 ppm, equal to 45 mg/kg bw per day (dog); inhibition of brain glutamine synthetase	Not neurotoxic Brain glutamine synthetase was not inhibited in dogs at 1000 ppm, equal to 58 mg/kg bw per day
Developmental neurotoxicity NOAEL	200 ppm, equal to 14 mg/kg bw per day (rat); increased motor activity, hippocampal pathology	No data	No data
Medical data			
	(Suicidal) poisonings producing several neurological effects and deaths. No adverse effects reported in plant production personnel.	No data	No data

Summary for glufosinate-ammonium

	Value	Study	Safety factor
ADI	0–0.01 mg/kg bw	Short-term studies (dog)	100
ARfD	0.01 mg/kg bw	Short-term studies (dog)	100

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MCPA

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Explanation

MCPA is the International Organization for Standardization (ISO)–approved common name for 4-chloro-*o*-tolyloxyacetic acid (International Union of Pure and Applied Chemistry) (Chemical Abstracts Service [CAS] No. 94-76-6). MCPA is a selective, systemic, hormone-type herbicide belonging to the phenoxyacetic acid family. It is used to control annual and perennial weeds in cereals, grassland and turf.

MCPA has not previously been evaluated by the Joint FAO/WHO Meeting on Pesticide Residues and was reviewed by the present Meeting at the request of the Codex Committee on Pesticide Residues.

There are several different formulations of MCPA, including the 2-ethylhexyl ester (2-EHE) (CAS No. 29450-45-1) and dimethylamine (DMA) (CAS No. 2039-46-5) salt of MCPA. Data on these formulations and, where appropriate, data on the sodium and potassium salts are included in this monograph, because both convert to MCPA ion in the mammalian digestive tract prior to absorption.

On this basis, doses of MCPA 2-EHE and MCPA DMA administered to laboratory animals are expressed as MCPA acid equivalents, calculated using their molecular weights (312.5 and 245.7, respectively) relative to the parent MCPA (200.6).

Unless otherwise stated, the contemporary unpublished studies evaluated in this monograph were performed by laboratories that were certified for good laboratory practice (GLP) and that complied, where appropriate, with the relevant Organisation for Economic Co-operation and Development (OECD) test guidelines or similar guidelines of the European Union or United States Environmental Protection Agency. As these guidelines specify the clinical pathology tests normally performed and the tissues normally examined, only significant exceptions to these guidelines are reported here, to avoid repetitive listing of study parameters. The studies in humans were conducted in accordance with the principles of good clinical practice and the Declaration of Helsinki, or equivalent statements prepared for use by national and/or multinational authorities.

The chemical structures of MCPA, MCPA 2-EHE and MCPA DMA are given in Figure 1.

Figure 1. Chemical structures of MCPA, MCPA 2-EHA and MCPA DMA

MCPA MCPA 2-EHE MCPA DMA

O OH Cl O CH₃ H₃C Cl CH₃ CH₃ H₂N CH₃ Cl CH₃

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution, metabolism and excretion

Rats

In a study predating GLP principles and contemporary test guidelines, phenyl-labelled [^{14}C]MCPA (radiochemical purity 97%) in 1% weight per volume (w/v) carboxymethylcellulose was administered by gavage to groups of fasted male and female Sprague-Dawley (SD) rats (CD strain). Following a single gavage [^{14}C]MCPA dose of 100 mg/kg body weight (bw) (three rats of each sex), radioactivity was excreted predominantly in urine (approximately 95% and 86% of the administered dose in males and females, respectively), with relatively low levels eliminated in faeces (6.2% and 5.6% in males and females, respectively). The majority of this radioactivity was eliminated within 48 hours of dosing (approximately 78% in urine and 5% in faeces), with the remainder excreted from 48 to 192 hours. The urine of two rats of each sex that contained the highest levels of radioactivity was subjected to metabolite analysis using thin-layer chromatography (TLC). The main metabolites were MCPA (approximately 71%) and one other undefined fraction (approximately 14%). Three minor fractions were also detected (approximately 5%, 4% and 3%, respectively).

The excretion of radioactivity in bile, urine and faeces was analysed in three male bile duct–cannulated rats following a single gavage [^{14}C]MCPA dose of 100 mg/kg bw. Recovery of radioactivity was low (mean of 34.5%), which the authors attributed to the poor condition of the animals. The mean levels of radioactivity in 0- to 24-hour bile, urine and faeces were 3.7%, 20.3% and 10.5%, respectively.

In a tissue distribution experiment, 14 rats of each sex received a single gavage dose of [^{14}C]MCPA at 100 mg/kg bw, with two rats of each sex sacrificed at various times to 192 hours for the analysis of radioactivity in plasma and tissues. The highest plasma and tissue concentrations of radioactivity were detected at the earliest sampling time (3 hours) and declined thereafter. At 3 and 6 hours after dosing, plasma contained the highest concentration of radioactivity. At later sampling

times, the carcass, skin, fat and adrenals had higher levels of radioactivity compared with plasma. In a separate experiment, whole-body autoradiography of three rats of each sex that received a single gavage dose of [^{14}C]MCPA at 100 mg/kg bw and were analysed at 6, 24 and 48 hours (one rat of each sex) confirmed this pattern of tissue distribution of radioactivity. In a further tissue distribution experiment, 10 rats of each sex received up to 14 consecutive daily gavage doses of [^{14}C]MCPA at 1 mg/kg bw. Two rats of each sex were sacrificed on days 1, 5, 10, 14 and 18. The pattern of uptake, tissue distribution and elimination of radioactivity was similar to that observed in the preceding single-dose studies (Gilbert & Hopkins, 1978).

In a published study by Tynnelä, Elo & Ylatilo (1990), MCPA (purity > 98%) in propylene glycol was administered to three or four fasted male SD rats as a single gavage dose of 475 mg/kg bw. Separate groups of rats were dosed concurrently with 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) at 238 or 475 mg/kg bw or 2,4-dichlorophenoxyacetic acid (2,4-D) at 300 mg/kg bw. Three hours after dosing, rats received a single intravenous injection of phenyl-labelled [^{14}C]MCPA. One hour later, blood and cerebrospinal fluid were collected, and the brain was dissected for the analysis of radioactivity by liquid scintillation counting (LSC). Plasma protein binding was also analysed. Lethargy and myotonia were observed following dosing with MCPA. Radioactivity was detected in blood, cerebrospinal fluid and all analysed brain regions (cerebral cortex, striatum, medulla oblongata, cerebellum and midbrain). The tissue to plasma ratio of radioactivity was approximately 1:8. Binding of MCPA to plasma proteins was observed (4–43% protein-unbound fraction).

Phenyl-labelled [^{14}C]MCPA (radiochemical purity 99.3%) was administered to male and female Wistar rats (Crl:(WI)BR strain) (five of each sex per group) as a single gavage dose of 5 or 100 mg/kg bw in 1% w/v carboxymethylcellulose. A separate group of rats (five of each sex per group) received 14 daily gavage doses of unlabelled MCPA (purity 99.7%) at 5 mg/kg bw followed by a single gavage dose of [^{14}C]MCPA at 5 mg/kg bw. Rats were fasted for 4 hours prior to and after dosing. Urine and faeces were collected at various times to 168 hours after administration of the radiolabelled dose. Rats were sacrificed at 96 hours (5 mg/kg bw dose) or 168 hours (100 mg/kg bw dose) after dosing, and various tissues were sampled. Radioactivity was analysed in excreta and tissues by LSC. Metabolites of MCPA were analysed in urine and faeces by TLC, high-performance liquid chromatography (HPLC) and liquid chromatography–mass spectrometry (LC-MS) using appropriate standards; samples were analysed with and without acid and alkaline hydrolysis and deconjugation with β-glucuronidase and arylsulfatase. In a pharmacokinetics experiment, rats received a single gavage dose of [^{14}C]MCPA at 5 or 100 mg/kg bw, and blood was collected at various times to 168 hours and then analysed for radioactivity; standard pharmacokinetic parameters were calculated for total radioactivity. In an additional tissue distribution experiment, groups of 12 rats of each sex received a single gavage dose of [^{14}C]MCPA at 5 mg/kg bw; at 1, 3 and 6 hours after dosing, four rats of each sex were sacrificed, and various tissues were analysed for radioactivity.

No signs of toxicity were reported.

The results of the excretion/mass balance experiment are summarized in Table 1. Recovery of radioactivity was greater than 95%. Irrespective of the dose or dosing regimen, the kidneys were the main route of excretion and accounted for greater than 93% of the administered dose in males and greater than 88% of the administered dose in females (urine plus cage wash); the majority of excretion occurred with 24 hours of dosing. Based on the level of radioactivity in urine and the cage wash, gastrointestinal absorption is estimated to be at least 95%. The faeces were a minor route of elimination, becoming more prominent in high-dose females, for which faecal excretion attained 20% of the administered dose in two of five rats. During a pilot study involving two male rats from the repeated-dose group, no radioactivity was detected in expired air.

The distribution of radioactivity in selected tissues of rats sacrificed at the end of the excretion/mass balance study (96 or 168 hours) is summarized in Table 2. The highest level of radioactivity was detected in fat, followed by the skin and carcass; the level of residual radioactivity

in the carcass was less than 0.8% of the administered dose. The slight retention of radioactivity in the ovaries and uterus of high-dose females was assumed by the study authors to be associated with visceral fat. Radioactivity was not detected in bone, brain, heart, lung, spleen, stomach contents or the thyroid.

Table 1. Mass balance in rats following oral dosing with [^{14}C]MCPA

Sample	Mean % of administered radioactive dose					
	5 mg/kg bw (single dose)		100 mg/kg bw (single dose)		5 mg/kg bw (repeated dose)	
	Males	Females	Males	Females	Males	Females
Urine						
- 6 h	32.9	24.8	16.9	8.1	27.9	36.1
- 12 h	39.5	39.9	43.6	37.1	35.7	31.2
- 24 h	3.2	8.9	19.6	16.8	13.6	9.7
- 48 h	1.0	1.8	3.9	7.4	1.9	1.7
- 72 h	0.2	0.3	1.2	1.9	0.3	0.3
- 96 h	0.1	0.2	0.6	1.2	0.1	0.1
- 120 h	—	—	0.3	0.5	—	—
- 144 h	—	—	0.2	0.3	—	—
- 168 h	—	—	0.2	0.3	—	—
Urine total	76.9	75.8	86.4	73.5	79.5	79.8
Cage wash	16.2	19.3	10.5	13.8	17.5	24.5
Urine + cage wash	93.3	95.4	97.2	88.3	97.4	104.6
Faeces	2.0	2.3	4.0	11.9	4.9	3.8
Total	95.1	97.7	101.1	100.1	102.3	109.0

From Jahanshahi & Stow (1995)
—, not analysed

Table 2. Tissue distribution of radioactivity in rats following oral dosing with [^{14}C]MCPA

Tissue	Mean concentration (µg [^{14}C]MCPA Eq/g tissue)					
	5 mg/kg bw (single dose)		100 mg/kg bw (single dose)		5 mg/kg bw (repeat dose)	
	Males	Females	Males	Females	Males	Females
Carcass	0.037	0.042	0.839	1.931	0.069	0.056
Skin	0.074	0.091	2.374	3.647	0.070	0.055
Plasma	0.002	0.005	0.345	0.923	0.005	ND
Blood	ND	ND	0.129	0.477	0.226	0.014
Fat	0.074	0.107	7.108	16.32	0.025	0.033
Skeletal muscle	ND	ND	0.083	0.059	ND	ND
Liver	0.026	ND	0.596	0.385	0.048	ND
Kidney	0.036	0.034	0.637	1.718	0.049	0.032
Stomach	ND	ND	0.109	0.506	0.021	ND
Gonads	ND	0.031	0.099	8.198	ND	ND
Uterus	NA	0.008	NA	6.164	NA	ND
Adrenals	ND	ND	ND	0.732	ND	ND

From Jahanshahi & Stow (1995)

Eq, equivalent; NA, not applicable; ND, not detected

Toxicokinetic parameters are summarized in Table 3. Maximum plasma concentrations (C_{max}) of radioactivity were achieved by about 2.5 hours (T_{max}), taking somewhat longer in high-dose females (approximately 4 hours). Plasma elimination half-lives were about 10–20 hours in males and about 20–30 hours in females; these values are discordant with the mass balance data indicating that the majority of radioactivity was excreted in the urine by 12 hours (Table 1). At both doses, the majority of plasma radioactivity showed an initial "rapid" decline to 24 or 48 hours (5 and 100 mg/kg bw, respectively), with subsequent levels declining more slowly to 168 hours. At both doses, a small number of rats still had detectable levels of radioactivity in plasma at the end of the experiment.

Table 3. Toxicokinetic parameters in rats following a single oral dose of [^{14}C]MCPA

Parameter	Mean values[a]			
	5 mg/kg bw		100 mg/kg bw	
	Males	Females	Males	Females
C_{max} (µg Eq/g)	26.8	33.2	373.1 (14)	357.8 (11)
T_{max} (h)	2.7	2.4	2.4	4.2
$t_{½}$ (h)	19.2	34.1	10.6	20.9
$AUC_{(0-\infty)}$ (µg Eq·h/g)	210	250	5180 (25)	5628 (23)

From Jahanshahi & Stow (1995)

AUC, area under the plasma concentration–time curve; C_{max}, maximum concentration in plasma; Eq, equivalent; $t_{½}$, half-life; T_{max}, time to reach C_{max}

[a] Values in parentheses represent the overall fold increase relative to the low dose.

In the additional tissue distribution experiment, radioactivity was detected in all analysed tissues at 1, 3 and 6 hours after a single dose of [^{14}C]MCPA at 5 mg/kg bw. The concentration of radioactivity in all tissues declined over time. With the exception of the stomach, stomach contents and kidneys, the concentration of radioactivity in all tissues was lower than that in plasma and/or blood. Other than the ovaries and testes, there were no apparent sex-related differences in the distribution pattern of radioactivity.

Analysis of pooled urine (0–6, 6–12 and 12–24 hours) from the excretion/mass balance experiment by TLC detected parent MCPA and 4-chloro-2-hydroxymethyl-phenoxyacetic acid (HMCPA). At the low single or repeated dose, urinary MCPA accounted for about 20–31%, 28–37% and 3–12% (51–80% in total) of the administered dose at 0–6, 6–12 and 12–24 hours, respectively. At this same dose, HMCPA accounted for approximately 4–7%, 2–7% and 0.2–2% (6–16% in total) of the administered dose at 0–6, 6–12 and 12–24 hours, respectively. At the high single [^{14}C]MCPA dose of 100 mg/kg bw, a somewhat larger proportion of urinary radioactivity was attributable to HMCPA at 0–6 hours, with a concomitant reduction in the proportion of MCPA. However, at 6–12 and 12–24 hours, the proportion of MCPA/HMCPA was consistent with that seen at 5 mg/kg bw. HPLC confirmed the proportions of MCPA and HMCPA in urine.

Some urine samples contained low levels of two minor unidentified metabolites (< 0.5% of the administered dose). Pretreatment of urine samples with β-glucuronidase or arylsulfatase prior to TLC or HPLC did not lead to the detection of glucuronide or sulfate conjugates of MCPA or its metabolites. Acid pretreatment of urine samples yielded a non-polar region coincident with anisole and reduced the level of HMCPA while increasing that of MCPA. Pretreatment of urine samples with alkali did not lead to the detection of any alkali-sensitive conjugates.

TLC of pooled (0- to 24-hour) faecal samples collected from rats that received a single 100 mg/kg bw dose of [^{14}C]MCPA identified MCPA and HMCPA (approximately 2% and 0.5% of the administered dose, respectively, in males; approximately 7% and 1.3% of the administered dose, respectively, in females). Three other regions representing unknown species were also reported.

HPLC of these same samples did not confirm the presence of HMCPA; the most abundant region was an unknown species (approximately 2% of the administered dose in males and 8.3% in females), followed by MCPA (approximately 1.1% of the administered dose in males and 2.2% in females). Some changes in the distribution of radioactivity were noted following pretreatment of faecal samples with β-glucuronidase or arylsulfatase, acid or alkali, but no definitive conclusions could be made by the study authors on the nature of these species.

Analysis of urine and faeces by LC-MS confirmed the presence of MCPA and HMCPA. In urine, a glycine conjugate of MCPA was identified, whereas two minor unidentified regions were also detected in faeces (Jahanshahi & Stow, 1995).

The proposed metabolic pathway of MCPA in rats is presented in Figure 2. MCPA is metabolically relatively stable but can be metabolized to HMCPA (via the C-oxidation of the 2-methyl moiety of MCPA) or by glycine conjugation.

Figure 2. Proposed metabolic pathways of MCPA, MCPA 2-EHE and MCPA DMA in rats

MCPA (purity 97.0%) was admixed in the diet at a concentration of 125, 250, 500, 1000, 1500 or 2200 parts per million (ppm) and offered to groups of four male Wistar rats (CrlGlxBrlHan:WI strain) for 14 days (equivalent to nominal doses of 10, 20, 40, 80, 120 and 180 mg/kg bw per day, respectively). On day 15, the diet was replaced with untreated diet, and rats were administered a single gavage dose of phenyl-labelled [^{14}C]MCPA (radiochemical purity 98.3%) in aqueous 0.5% w/v carboxymethylcellulose at an equivalent dose. Blood was sampled at 1, 2, 4, 8, 12, 24 and 48 hours after dosing and analysed for radioactivity by LSC; standard toxicokinetic parameters were determined. Rats were offered diets containing unlabelled MCPA from 24 to 48 hours.

There were no treatment-related clinical signs. The body weight gain of high-dose rats was about 45–75% lower than that of all other groups from day 0 to day 14. With the exception of the

high-dose diet, achieved doses were higher than the nominal doses during weeks 1 and 2 (17–54% and 14–24% higher, respectively).

Toxicokinetic parameters are summarized in Table 4. At 120 and 180 mg/kg bw, the time to C_{max} (T_{max}) was 2–8 times higher than at lower doses, which, in association with the under-proportional increase in C_{max} from 120 to 180 mg/kg bw, led the authors to suggest that gastrointestinal absorption might be delayed at these higher doses. Alternatively, the higher T_{max} at 120 and 180 mg/kg bw might reflect the achievement of equilibrium between absorption and excretion. Following attainment of C_{max}, radioactivity showed a continuous decline over time, with three different phases identified by the authors at every dose: phase 1—mixed absorption/excretion phase (approximately 0–12 hours); phase 2—excretory phase (12–24 hours); and phase 3—mixed absorption/excretion, where rats were redosed with non-labelled MCPA (24–48 hours). Up to 80 mg/kg bw, there was a generally linear increase in the area under the plasma concentration–time curve (AUC). From 120 to 180 mg/kg bw, the AUC increased by a greater proportion than the dose increment, particularly at 12–24 hours (the excretory phase), suggesting saturation of excretion. Initial plasma half-lives calculated from plasma concentration–time curves during phase 2 were consistent from 10 to 80 mg/kg bw, but thereafter increased markedly. While not a reliable indicator of the saturation of excretion, these initial half-lives support the AUC results—namely, that excretion is saturated at doses greater than 80 mg/kg bw. Plasma concentrations of radioactivity at 24 hours after dosing (which can be taken to represent the carry-over to the next dosing day) increased by a greater proportion than the dose increment at and above 80 mg/kg bw. The carry-over at 120 and 180 mg/kg bw was relatively large and would likely lead to an increased body burden on subsequent treatment days. The kinetics suggested that this phenomenon would be progressive over repeated dosing regimens at high oral doses. On the basis of these findings, the authors concluded that doses greater than 80 mg/kg bw per day may result in progressive toxicity in rats (Beimborn & Leibold, 2003a).

Table 4. Toxicokinetic parameters in rats following repeated dietary exposure to MCPA followed by a single dose of [^{14}C]MCPA

Parameter	Mean values[a]					
	10 mg/kg bw	20 mg/kg bw	40 mg/kg bw	80 mg/kg bw	120 mg/kg bw	180 mg/kg bw
T_{max} (h)[b]	1	2	4	2	8	8
C_{max} (µg Eq/g)	39	70 (1.8)	130 (4.3)	201 (5.2)	250 (6.4)	324 (8.3)
$AUC_{0-\infty}$ (µg Eq·h/g)	367	634 (1.7)	1243 (3.4)	2394 (6.5)	5743 (16)	11 185 (30.5)
$AUC_{12-14\,h}$ (µg Eq·h/g)	66	75 (1.1)	145 (2.2)	543 (8.2)	2138 (32.4)	3226 (48.9)
Plasma concentration after 24 h (µg Eq/g)	0.56	0.96 (1.7)	1.76 (3.1)	4.93 (8.8)	120.08 (214)	237.28 (424)
$t_{½}$ (h)						
- phase 1	10.55	13.47	18.46	34.64	—	—
- phase 2	2.89	2.93	2.87	3.08	14.43	35.48
- phase 3	16.63	12.74	15.30	14.95	5.91	13.63

From Beimborn & Leibold (2003a)

AUC, area under the plasma concentration–time curve; C_{max}, maximum concentration in plasma; Eq, equivalent; $t_{½}$, half-life; T_{max}, time to reach C_{max}

[a] Values in parentheses represent the overall fold increase relative to the low dose.

[b] Observed value.

Groups of 12 time-mated female Wistar rats (CrlGlxBrlHan:WI) were administered daily gavage doses of unlabelled MCPA (purity 97.0%) at 20, 40, 60, 80 or 120 mg/kg bw per day in 0.5% w/v aqueous carboxymethylcellulose from days 6 to 18 post-coitum. On day 19, rats were administered a single gavage dose of phenyl-labelled [^{14}C]MCPA (radiochemical purity 98.3%) in aqueous 0.5% w/v carboxymethylcellulose at an equivalent dose. Three dams per group were sacrificed at 4, 8, 12 and 24 hours after this dose, and blood was collected. Samples of fetal blood (pooled by dam) and amniotic fluid were also taken at these same times. Total radioactivity was analysed by LSC, and the results were used to calculate AUC values.

The concentration of radioactivity in plasma (dams and fetuses) and amniotic fluid was maximal at 4 hours and rapidly declined thereafter. At 80 and 120 mg/kg bw, the maximum concentration of radioactivity was attained at about 8 hours, and the concentration also showed a rapid decline thereafter. In maternal and fetal plasma and amniotic fluid, AUCs indicated two phases of decline: phase 1—mixed absorption/excretion phase (0–12 hours after dosing); and phase 2—excretory phase (12–24 hours after dosing). The concentration of radioactivity in fetal plasma and amniotic fluid was approximately 3- to 7-fold and 4- to 9-fold lower, respectively, than that in maternal plasma. In both fetal plasma and amniotic fluid, $AUC_{4–24\,h}$ increased by greater than the dose increment at and above 40 mg/kg bw, whereas in maternal plasma, the increase was proportional (Table 5) (Beimborn & Leibold, 2003b).

Table 5. $AUC_{4–24\,h}$ values in plasma and amniotic fluid

Parameter	Mean $AUC_{4–24\,h}$ (µg Eq·h/g)[a]				
	20 mg/kg bw	40 mg/kg bw	60 mg/kg bw	80 mg/kg bw	120 mg/kg bw
Maternal plasma	612	1057 (1.7)	1660 (2.7)	2556 (4.2)	3653 (6)
Fetal plasma	89	202 (2.3)	421 (4.8)	813 (9.2)	1315 (14.8)
Amniotic fluid	65	162 (2.5)	288 (4.4)	419 (6.4)	820 (12.6)

From Beimborn & Leibold (2003b)
AUC, area under the plasma concentration–time curve; Eq, equivalent
[a] Values in parentheses represent the overall fold increase relative to the low dose.

In a pharmacokinetics study, phenyl-labelled [^{14}C]MCPA 2-EHE (radiochemical purity 99.6%) or phenyl-labelled [^{14}C]MCPA DMA (radiochemical purity 98.5%) was administered to male Wistar rats (Crl:(WI)BR strain) (five per group) as a single gavage dose of 5 mg/kg bw in 1% w/v carboxymethylcellulose. Blood was sampled at various times to 168 hours after dosing. In an absorption, distribution, metabolism and excretion study conducted under the same conditions, urine and faeces were collected at various times to 168 hours after dosing. Expired air was collected to 48 or 72 hours (MCPA 2-EHE and MCPA DMA, respectively). Rats were sacrificed at 168 hours after dosing, and various tissues were sampled. Radioactivity was quantified in plasma, excreta and tissues by LSC. Metabolites were analysed in urine and faeces by TLC and HPLC, and their identity was confirmed by LC-MS. Samples were also subjected to glucuronidase or aryl sulfatase digestion or to acid hydrolysis.

There were no signs of toxicity, and rats gained body weight normally over the duration of the study. Pharmacokinetic parameters for MCPA 2-EHE and MCPA DMA were comparable (Table 6), with radioactivity detected up to 72 and 48 hours after dosing, respectively. These parameters are consistent with those determined for MCPA in the study by Jahanshahi & Stow (1995).

Table 6. Plasma pharmacokinetic parameters in male rats following a single oral dose of [^{14}C]MCPA 2-EHE or [^{14}C]MCPA DMA

Parameter	Mean value	
	5 mg/kg bw MCPA 2-EHE	5 mg/kg bw MCPA DMA
C_{max} (µg Eq/g)	26	24.1
T_{max} (h)	3.4	2.4
$t_{½}$ (h)	7.7	6.3
$AUC_{0-\infty}$ (µg Eq·h/g)	247.3	215.5

From Burke (1995)
AUC, area under the plasma concentration–time curve; C_{max}, maximum concentration in plasma; Eq, equivalent; $t_{½}$, half-life; T_{max}, time to reach C_{max}

The results of the excretion/mass balance study are summarized in Table 7. Mean recovery of radioactivity was approximately 102% following dosing with [^{14}C]MCPA 2-EHE and approximately 104% following dosing with [^{14}C]MCPA DMA. Following dosing with either compound, about 80% of the administered radioactivity was detected in urine within 12 hours, and 89% over 168 hours. Based on the levels of radioactivity in urine and the cage wash, gastrointestinal absorption was greater than 99%. The faeces were a minor route of elimination, accounting for approximately 2% and 4% of the administered dose of MCPA 2-EHE and MCPA DMA, respectively. Radioactivity was detected in the faeces of rats for up to 72 hours after dosing with MCPA 2-EHE and for up to 72–168 hours after dosing with MCPA DMA. No radioactivity was detected in expired air.

Table 7. Mass balance in rats following oral dosing with [^{14}C]MCPA 2-EHE or [^{14}C]MCPA DMA

Parameter	Mean % of the administered radioactive dose	
	5 mg/kg bw MCPA 2-EHE	5 mg/kg bw MCPA DMA
Urine		
- 0–6 h	15.5	13.5
- 6–12 h	63.1	67.9
- 12–24 h	7.4	6.3
- 24–48 h	2.2	0.93
- 48–72 h	0.26	0.20
- 72–96 h	0.11	0.10
- 96–120 h	0.050	0.073
- 120–144 h	0.046	0.046
- 144–168 h	0.028	0.038
Urine total	88.72	88.99
Cage wash	11.43	10.85
Urine + cage wash	100.15	99.84
Faeces	1.71	4.101
Carcass	0.33	ND
Total	102.2	104.0

From Burke (1995)
ND, not detected

At 168 hours after dosing, radioactivity was detected in the skin (mean of 0.067 and 0.056 µg Eq/g for MCPA 2-EHE and MCPA DMA, respectively), fat (mean of 0.007 and 0.033 µg Eq/g for MCPA 2-EHE and MCPA DMA, respectively) and kidney (0.010 µg Eq/g for MCPA DMA), but was

below the limit of detection for all other analysed tissues (adrenals, blood, brain, spleen, stomach, muscle, bone, plasma, stomach contents, gonads and heart).

No parent compounds were detected in urine or faeces. The major urinary metabolite was MCPA (approximately 70–79% of the administered dose), followed by HMCPA (approximately 13–15% of the administered dose) and the glycine conjugate of MCPA, which was not quantifiable due to its similar chromatographic properties to MCPA. No glucuronide or sulfate conjugates of MCPA or its metabolites were detected, whereas acid pretreatment did not lead to the detection of any acid-sensitive conjugates. In faeces, HMCPA and MCPA were detected (0.3–3.6% and 0.1–1.5% of the administered dose, respectively). The proposed metabolic pathways for MCPA 2-EHE and MCPA DMA are presented in Figure 2. In the digestive tract, MCPA 2-EHE undergoes ester hydrolysis, whereas MCPA DMA dissociates to yield MCPA ion. Following absorption, MCPA may undergo C-oxidation to yield HMCPA or may be glycine conjugated (Burke, 1995).

In a published study, the renal handling of MCPA was investigated in Wistar rats (Uje:WIST strain) under conditions of forced diuresis (with frusemide, 5 mg/kg bw, intraperitoneal), an oral water load (50 ml/kg bw), sodium bicarbonate administration (50 ml/kg bw, intraperitoneal administration of a 2% solution), when renal tubular transport was blocked (with probenecid, 200 mg/kg bw, intraperitoneal; or *p*-aminohippurate, 2.5 g/kg bw, intraperitoneal) or in the presence of triiodothyronine (T_3) (200 µg/kg bw intraperitoneally for 3 days). In the in vivo phase of the study, [^{14}C]MCPA (unspecified position of label) was mixed with unlabelled MCPA (purity > 99%) at 0.1–0.25% of the total dose in 0.9% sodium chloride and administered as a single intraperitoneal dose of 0.5, 5, 10 or 20 mg/kg bw to six rats per group. Urine was sampled at 1 and 2 hours after dosing for the analysis of MCPA by LSC. Plasma concentrations of MCPA were also analysed from 0.5 to 24 hours following a single intraperitoneal injection of MCPA at 5 or 10 mg/kg bw.

Renal excretion of MCPA was about 50% of the administered dose 5 hours after dosing and about 60% 10 hours after dosing at 0.5–10 mg/kg bw. At the highest dose of MCPA (i.e. 20 mg/kg bw), the level of renal excretion was decreased (approximately 20% of the administered dose). Maximum plasma concentrations of MCPA were 28 and 54 µg/ml at 5 and 10 mg/kg bw, respectively. The plasma half-life was approximately 8–10 hours, whereas a low volume of distribution (17% or 18%) was calculated. At plasma concentrations of MCPA up to 80 µg/ml, binding to plasma albumin was 85%; this high degree of binding would limit the tissue distribution of MCPA. Renal excretion of MCPA was increased 3-fold following the inhibition of tubular reabsorption by an oral water load or bicarbonate administration. An approximately 25% reduction in renal excretion was achieved following simultaneous administration of probenecid or *p*-aminohippurate and MCPA. Repeated administration of T_3 significantly ($P < 0.05$) increased the excretion of MCPA.

In the in vitro phase of the study, the accumulation of MCPA in renal cortical slices was investigated at concentrations ranging from 10^{-8} to 10^{-3} mol/l under aerobic (oxygen) or anaerobic (nitrogen) conditions. The accumulation of MCPA was reduced under anaerobic conditions.

The authors concluded that the observations suggested active tubular transport of MCPA, with renal excretion increased by inhibiting such tubular reabsorption (Bräunlich et al., 1989).

Dogs

Phenyl-ring labelled [^{14}C]MCPA (radiochemical purity 93.8%) was administered to four male Beagle dogs as a single oral dose of 5 mg/kg bw in gelatine capsules. Blood, urine and faeces were sampled at various times to 120 hours after dosing. Radioactivity was quantified by LSC. Urinary and faecal metabolites were analysed by HPLC and LC-MS. The same four dogs were then redosed (after an unspecified washout period) with [^{14}C]MCPA at 100 mg/kg bw under the same experimental conditions.

No toxicological signs were observed during the course of the study.

Excretion/mass balance results are summarized in Table 8. Recovery of radioactivity was approximately 79% and 85% following dosing at 5 and 100 mg/kg bw, respectively. At 5 mg/kg bw,

radioactivity was excreted mainly in the urine, accounting for about 58% of the administered dose, with approximately 17% of the administered radioactivity eliminated in faeces. At 100 mg/kg bw, a greater proportion of radioactivity was detected in faeces (approximately 49%) than in urine (34%). At both doses, elimination was incomplete by 120 hours, with about 4% and 2% of the dose still detectable in urine and faeces, respectively.

Table 8. Mass balance in dogs following a single oral dose of [^{14}C]MCPA

Sample	Mean % of the administered radioactive dose	
	5 mg/kg bw	100 mg/kg bw
Urine	58.4	34.0
Faeces	16.8	49.1
Cage wash	3.5	2.1
Cage debris	0.03	0.06
Total	78.7	85.2

From Hardwick (1999, 2000)

Pharmacokinetic data are summarized in Table 9. Radioactivity was detected in plasma within 1 hour of dosing and was maximal at 4.5 and 7.0 hours at 5 and 100 mg/kg bw, respectively. At 100 mg/kg bw, C_{max} and $AUC_{0-\infty}$ were about 10-fold higher than at 5 mg/kg bw. Between 19% and 32% of the AUC was extrapolated to be after the last time point, indicating that clearance of radioactivity was not complete at the final measurement, 120 hours after dosing. Concentrations of radioactivity in blood paralleled those in plasma but were approximately 2-fold lower, indicating that MCPA was substantially extracellular. The plasma half-life values were approximately 45 and 47 hours at 5 and 100 mg/kg bw, respectively.

Table 9. Plasma pharmacokinetic parameters in dogs following a single oral dose of [^{14}C]MCPA

Parameter	Mean value[a]	
	5 mg/kg bw	100 mg/kg bw
C_{max} (µg Eq/g)	35.95	341.2 (9.5)
T_{max} (h)	4.5	7.0
$t_{½}$ (h)	45.27	46.70
AUC_{0-t} (µg Eq·h/g)	2030	17 195 (8.5)
$AUC_{0-\infty}$ (µg Eq·h/g)	2539	20 454 (8.1)

From Hardwick (1999, 2000)

AUC, area under the plasma concentration–time curve; C_{max}, maximum concentration in plasma; Eq, equivalent; $t_{½}$, half-life; T_{max}, time to reach C_{max}

[a] Values in parentheses represent the overall fold increase relative to the low dose.

At both doses, four major radioactive regions were detected in urine by HPLC, the author noting the high level of inter-animal variation. At 5 mg/kg bw, MCPA and HMCPA accounted for 4.0–29.3% and 1.1–6.5% of the administered dose, respectively. At 100 mg/kg bw, MCPA and HMCPA accounted for 1.7–4.0% and 2.1–15.4% of the administered dose, respectively. The two other (unidentified) major regions of radioactivity respectively accounted for 12.1–37.6% and 5.1–10.6% of the administered dose at 5 mg/kg bw and 6.5–13.6% and 3.1–7.6% of the administered dose at 100 mg/kg bw. Urine samples taken at 12 and 28 hours after dosing with [^{14}C]MCPA at 100 mg/kg bw were subjected to LC-MS. The presence of MCPA (29.3% of the administered dose) and HMCPA (1.1–6.5% of the administered dose) was confirmed. Glycine and taurine conjugates of MCPA were

also detected (12.1–37.6% and about 10% of the administered dose, respectively). An additional unidentified peak was also detected, which the author suggested was probably another conjugate of MCPA, but further characterization was not possible.

In faeces, three main radioactive peaks were detected by HPLC. One peak was identified as MCPA (5.8–9.6% of the administered dose at 5 mg/kg bw and 11.5–27.6% of the administered dose at 100 mg/kg bw), whereas the two other peaks were tentatively identified as the glycine and taurine conjugates of MCPA (0.5–1.5% and 1.8–4.2% of the administered dose, respectively, at 5 mg/kg bw and 0.7–4.4% and 0.9–18.8%, respectively, at 100 mg/kg bw). HMCPA was not identified in faeces. The presence of conjugated MCPA in the faeces of dogs indicated biliary excretion to be significant in this species (Hardwick, 1999, 2000).

Timchalk (1998, 2004) compared published toxicokinetic parameters (volume of distribution, renal clearance and half-life) for MCPA (and 2,4-D) in the rat, dog and human using allometric scaling on the basis of body weight (Table 10). Volume of distribution scaled as a function of body weight and was only marginally larger than blood volume. In the dog, half-life and renal clearance of MCPA did not scale as a function of body weight, whereas for rats and humans, these parameters were in reasonable agreement with allometric scaling. Longer half-lives and slower elimination in the dog result in substantially higher body burden at comparable nominal doses relative to other species. Proposed mechanisms for reduced clearance in the dog include saturation of renal clearance and increased renal tubule reabsorption. On the basis of these comparisons, the author suggested that it is inappropriate to extrapolate observations of toxicity induced by MCPA (or other phenoxyacetic acid herbicides) in the dog to humans because of the limited capacity of the dog to excrete organic acids relative to other species.

Table 10. Interspecies comparison of toxicokinetic parameters for MCPA

Parameter	Rat	Dog	Human
Body weight (kg)	0.25	13	70
Dose of MCPA (mg/kg bw)	5	5	0.015
V_d (l)	0.04	1.81	5.25
Renal clearance (ml/h)	3.7	12	132
$t_{½}$ (h)	5.8	46	11

From Timchalk (1998, 2004)
$t_{½}$, half-life; V_d, volume of distribution

Humans

In a non-guideline study predating the most recent Declaration of Helsinki, Fjeldstat & Wannag (1977) investigated the urinary excretion of MCPA to ascertain whether it could be used to estimate MCPA exposure. A single 5 mg dose of MCPA (unspecified purity and vehicle) was ingested by four healthy volunteers (32–36 years of age). Urine was collected prior to ingestion and then at various times to 5 days after dosing. Samples were hydrolysed with sulfuric acid prior to HPLC analysis. Blood was sampled at 2, 7, 12 and 24 hours and at 2, 3, 4 and 10 days after dosing for the analysis of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase, alkaline phosphatase (ALP), creatine kinase and gamma-glutamyl transpeptidase (GGT). Recovery was approximately 60% of the administered dose by 96 hours after dosing. The mean level of MCPA in urine was about 41%, 52%, 54% and 55% of the administered dose at 24, 48, 72 and 96 hours after dosing, respectively. Graphically presented data illustrated that urinary excretion was nearly completed by 48 hours. After 5 days, the concentration of MCPA in urine was below the limit of detection (0.2 µg/ml). There was no treatment-related effect on any of the analysed serum enzymes.

In a second non-guideline study also predating the most recent Declaration of Helsinki, five healthy volunteers (three males and two females) ingested a single oral dose of MCPA (as the potassium salt) (unspecified purity) in gelatine capsules at 15 µg/kg bw. Blood was sampled at 30 minutes and 1, 3, 6 and 24 hours; urine was sampled over 72 hours. The C_{max} of 0.15 µg/ml was measured at 1 hour after dosing, declining to 0.09, 0.07 and less than 0.04 µg/ml at 3, 6 and 22 hours, respectively. Based on these concentrations, the elimination half-life from plasma is approximately 6 hours. Urinary excretion was the highest over the first 6 hours after dosing (approximately 0.45 µg/minute), declining to 0.08 µg/minute by 48 hours. Approximately 40% of the administered dose was excreted within 24 hours. Urine collected from one subject was reported to contain 56–73% conjugated MCPA, although the identity of the conjugated species was not described (Kolmodin-Hedman et al., 1983).

Kolmodin-Hedman et al. (1983) also examined the dermal absorption and urinary excretion of MCPA in the same volunteers (presumably after a washout period). In the first experiment, the volunteers dipped their hands and forearms into a 2% aqueous solution of MCPA, allowed them to dry and then washed them with soap and water. Blood was sampled at 30 minutes and 1, 3, 6 and 24 hours; urine was sampled over 72 hours. No MCPA was detectable in blood or urine.

In a second experiment, about 1 g of MCPA (prepared from a 75% liquid formulation) was applied to 10 cm^2 of the thigh via an occlusive dressing. After 2 hours, the dressing was removed and the application site washed with soap and water. Blood was sampled at 30 minutes and 1, 3, 6 and 24 hours; urine was sampled over 72 hours. The experiment was repeated, with blood and urine sampled up to 55 hours and 7 days, respectively. No clinical signs were observed. The plasma concentration of MCPA was variable across the five volunteers. In two volunteers, MCPA was not detectable after 3 hours. In the other three volunteers, plasma concentrations of MCPA increased slowly, reaching a maximum at about 24 hours after application and thereafter declining slowly; MCPA was still detectable after 55 hours. In urine, the concentration of MCPA was detected within 3 hours of dermal application, reaching a maximum at 24–48 hours and remaining detectable to 144–168 hours. The urinary half-life in two volunteers was 25 and 52 hours. The mean cumulative urinary excretion of MCPA was 1.73 mg after 6 days, accounting for approximately 0.002% of the applied amount of 1 g.

Roberts et al. (2011) examined the toxicokinetics and saturable protein binding of MCPA in patients with acute MCPA poisoning. The time to hospital admission was 2.5–6 hours (mean 3.3 hours) after poisoning, which was the T_{max} for 22 of 25 patients. C_{max} values were 3.7 hours in one of the remaining patients and 7 hours in the other two, suggesting prolonged absorption. At plasma concentrations above 200 mg/l, the apparent half-life was estimated to be 25.5 hours, whereas at lower concentrations, it was approximately 17 hours. Dose-dependent protein binding occurred, which was saturated when plasma concentrations of MCPA exceeded about 240 mg/l. Scatchard analysis suggested protein binding at two sites, with saturation of the high-affinity site occurring at 115 mg/l.

1.2 In vitro studies

The mechanism of intestinal uptake of MCPA was investigated in monolayers of the human colorectal adenocarcinoma epithelial cell line, Caco-2, in the presence of various metabolic inhibitors. At pH 6, uptake occurred within 5 minutes, with about a 5-fold lower uptake at pH 7.4. Uptake was biphasic, with an initial saturable carrier-mediated process at low concentrations and a non-saturable process (simple diffusion) at higher concentrations. Uptake was significantly ($P < 0.05$) inhibited at low temperature and in the presence of sodium azine, 2,4-dinitrophenol and carbonyl cyanide *p*-(trifluoromethoxy) phenylhydrazone. A number of monocarboxylic acids significantly ($P < 0.05$) inhibited uptake, with the most potent including benzoic acid, ferulic acid, salicylic acid and *p*-coumaric acid (30–40% of the control). The results suggested that MCPA uptake is via an H^+-linked monocarboxylic acid transporter (Kimura, Tsukagoshi & Endo, 2008).

In a subsequent study by Kimura et al. (2012), the uptake from apical membranes and apical to basolateral transport (i.e. secretory transport) of MCPA were investigated in monolayers of Caco-2 cells. Absorptive transport via an H^+-linked monocarboxylic acid transporter on the apical membranes of the monolayer was confirmed. Secretory transport was found to be mediated by a probenecid-sensitive transporter present on the basolateral side of the monolayer.

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

The results of acute toxicity tests of MCPA, MCPA 2-EHE and MCPA DMA administered to different animal species are summarized in Table 11. Clinical signs generally occurred within hours of dosing and included piloerection, apathy, hunched posture, abnormal gait, decreased respiration, ptosis, pallor and occasionally ataxia, twitching and tonic convulsions; survivors recovered within about 2 days.

(b) Dermal and ocular irritation

The results of skin and eye irritation tests on MCPA, MCPA 2-EHE and MCPA DMA conducted in rabbits are summarized in Table 12.

(c) Dermal sensitization

In a non-guideline study, MCPA (purity 94.8%) was analysed for its skin sensitization potential in the guinea-pig maximization test. Twenty female guinea-pigs were included in the test group, and 10 in the control group. Following the first and second challenges with 10% MCPA, two guinea-pigs showed very slight erythema or slight erythema and very slight oedema. On the basis of these findings, MCPA was classifiable as a non-skin sensitizer (Kirsch & Hildebrand, 1984).

In a non-guideline study, Grundler & Kirsch (1984) examined the skin sensitization potential of MCPA (purity 94.8%) in the guinea-pig open epicutaneous test. Groups of eight female guinea-pigs were induced with 1.5%, 5%, 15% or 50% MCPA and challenged twice thereafter with these same four concentrations across the four treated groups. In the 1.5% and 50% induction groups, very slight (barely perceptible) erythema was recorded in one of eight animals in each of the 1.5% and 50% induction groups following the first challenge. Following the second challenge, very slight (barely perceptible) erythema was recorded in the 1.5% induction group (4/8, 3/8 and 1/8 animals at challenge concentrations of 50%, 15% and 5%, respectively) and the 5% induction group (1/8 and 2/8 animals, respectively, at challenge concentrations of 50% and 15%, respectively). No oedema was observed in any animal following either challenge. MCPA was not classified as a skin sensitizer.

Using a modification of the Buehler method, Denton (1993a,b) examined the potential of MCPA 2-EHE (purity 93.9%) or MCPA DMA (purity 77.67%) to cause delayed contact hypersensitivity in the guinea-pig. There were 10 animals in both the control and test groups. No evidence of skin sensitization was recorded for either MCPA 2-EHE (Denton, 1993a) or MCPA DMA (Denton, 1993b).

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

In a 4-week range-finding study, MCPA (purity 94.8%) was admixed in the diet and fed ad libitum to groups of B6C3F1 mice (five of each sex per group) at a concentration of 0, 100, 300, 900 or 2700 ppm. The 300 ppm group consisted of four males and six females. The respective mean intakes of MCPA were 0, 20, 59, 177 and 696 mg/kg bw per day for males and 0, 24, 72, 208 and 765 mg/kg bw per day for females at 0, 100, 300, 900 and 2700 ppm. Blood was sampled at the end of the study for the analysis of haematology parameters and a limited number of clinical chemistry

parameters (creatinine, urea, ALT and ALP). Organ weights were recorded, and histopathology was undertaken on selected tissues. Only body weight data were statistically analysed.

Table 11. Results of studies of acute toxicity of MCPA and its salts

Species	Strain	Sex	Route	Purity (%)	Vehicle	LD_{50} or LC_{50} (mg/kg bw or mg/l)	Reference
MCPA acid							
Rat	Wistar	Male and female	Oral	Not specified	0.5% w/v aqueous CMC	1160	Kirsch & Hildebrand (1983a)
Rat	SD	Female	Oral	97.5	Arachis oil	~500 (ATC method)	Sanders (2006)[a]
Rat	Wistar	Male and female	Dermal	Not specified	0.5% w/v aqueous CMC	> 4000	Kirsch & Hildebrand (1983b)
Rat	Wistar	Male and female	Inhalation (4 h, head only)	94.8 MMAD = 5.2 µm Dust aerosol mixture	1% fumed silica	> 6.36	Klimisch et al. (1986)[a]
MCPA 2-EHE							
Rat	SD	Male and female	Oral	93.9	Water	1152 ae (male) 832 ae (female)	Allan (1992a)[a]
Rabbit	NZW	Male and female	Dermal	93.9	Water	> 2000	Denton (1992)[a]
Rat	SD	Male and female	Inhalation (4 h, whole body)	93.9 MMAD = 2.9 µm	Water	> 4.5	Jackson, Molloy & Hardy (1993a)[a]
MCPA DMA							
Rat	SD	Male and female	Oral	77.7	Water	820 ae	Allan (1992b)[a]
Rabbit	NZW	Male and female	Dermal	77.7	Water	> 2000	Allan (1992c)[a]
Rat	SD	Male and female	Inhalation (4 h, whole body)	77.7 MMAD = 2.2 µm	Water	> 4.72	Jackson, Molloy & Hardy (1993b)[a]

ae, acid equivalents; ATC, acute toxic class; CMC, carboxymethylcellulose; LC_{50}, median lethal concentration; LD_{50}, median lethal dose; MMAD, mass median aerodynamic diameter; NZW, New Zealand White; SD, Sprague-Dawley; w/v, weight per volume

[a] Statement of compliance with principles of good laboratory practice; guideline study.

There were no deaths. Clinical signs were confined to all high-dose mice and consisted of motor disturbances (drawing and convulsive lateral stretching of the hindlimbs; jerky motor activities), which commenced in the majority of mice on day 3 and persisted for the duration of the study. These disturbances were accompanied by other signs (squatting posture, closed eyelids and staggering gait), which had resolved in the majority of mice by day 10. In a swimming test designed to examine neuromuscular coordination and grip reflex, there was a delay in the beginning of swimming in all high-dose mice, but this had normalized after a few swimming movements.

Table 12. Results of studies of dermal and ocular irritation of MCPA and its salts in rabbits

Strain	Sex	Test material	Application site	Exposure period	Result	Reference
MCPA acid						
White Vienna	Male and female	50% w/w aqueous formulation	Abraded and intact skin	24 h, occluded	Primary irritation index = 2.5	Kirsch & Hildebrand (1983c)
NZW	Female	94.22% purity	Intact skin	4 h, semi-occluded	No reaction to treatment	Liggett (1992a)[a]
NZW	Male	97.5% purity	Intact skin	4 h, semi-occluded	No reaction to treatment	Pooles (2007)[a]
White Vienna	Male and female	~64 mg MCPA powder	Eye	—	Severe eye irritation (primary irritation index = 76)	Kirsch & Hildebrand (1983d)
MCPA 2-EHE						
NZW	Female	93.9% purity	Intact skin	4 h, semi-occluded	Slight skin irritation	Liggett (1992b)[a]
NZW	Male and female	93.9% purity	Eye	Eye unwashed for 24 h	Slight eye irritation	Liggett (1992e)[a]
MCPA DMA						
NZW	Male and female	77.67% purity	Intact skin	4 h, semi-occluded	Very slight skin irritation	Liggett (1992c)[a]
NZW	Female (*n* = 1)	77.67% purity	Eye	Eye unwashed for 24 h	Severe eye irritation[b]	Liggett (1992d)[a]
White Vienna	Male and female	1.6% aqueous solution	Eye	—	No irritation	Kieczka (1984)

NZW, New Zealand White; w/w, weight per weight

[a] Statement of compliance with principles of good laboratory practice; guideline study.

[b] Animal killed for humane reasons on day 7.

At 2700 ppm, body weight gain was significantly lower ($P < 0.01$) than the control values throughout the dosing period; all high-dose mice lost approximately 10–20% of their body weight during the study. At this same dietary concentration, feed consumption was lower than the control value from day 1 to day 7 (2.9 g versus 4.4 g in males and 2.7 g versus 3.9 g in females).

Perturbations in haematology and clinical chemistry parameters occurred at the highest dose. Relative to the control group, there was a 10–15% reduction in haemoglobin, haematocrit and erythrocyte counts in both sexes, a 45% (males) and 26% (females) reduction in platelets, an approximately 40% reduction in lymphocytes and an 8-fold (male) and 4-fold (female) increase in monocytes. ALT activity was increased 14-fold (males) and 39-fold (females), whereas ALP activity was increased 6-fold (males) and 8-fold (females). Reduced serum urea and creatinine concentrations were noted in high-dose females (but not males), which may have been a consequence of the body weight loss in this group rather than a direct effect of treatment. While there were no apparent differences in mean values between the control and 900 ppm groups, the authors reported isolated cases of increased ALT activity (one female) and decreased creatinine (one female), haemoglobin (two males and one female), haematocrit and erythrocytes (one male and one female) at 900 ppm. There were no treatment-related effects on any haematology or clinical chemistry parameter at 100 or 300 ppm.

Macroscopic examination revealed emaciation in all high-dose mice, with a loss of adipose tissue consistent with a peroxisome proliferating effect. The decrease in the weight or size of a

number of organs (adrenals, kidney, spleen, testes, ovaries and uterus) was attributable to this emaciation. Histopathology revealed lymphocyte depletion in the spleen, atrophy of the germinal epithelium of the testes and the absence of corpora lutea and glandular atrophy of the uterus consistent with cachexia. In contrast to other organs, liver weights were increased relative to the control at the highest dose (mean increase of 37% in males and 72% in females) in association with cloudy swelling of hepatocytes (all mice) and necrosis (4/5 males and 4/5 females). At 900 ppm, cloudy swelling of the hepatocytes was observed in one female. No treatment-related pathology was noted at lower doses.

The no-observed-adverse-effect level (NOAEL) was 300 ppm (equal to 59 and 72 mg/kg bw per day in males and females, respectively), based on reduced body weight gain and liver pathology at the next higher dose of 900 ppm (equal to 177 and 208 mg/kg bw per day in males and females, respectively) (Kirsch et al., 1985a).

Rats

In a non-guideline study, MCPA (purity 94.8%) was admixed in the diet and fed ad libitum to Wistar rats (15 of each sex per group) at a dietary concentration of 0, 50, 150 or 450 ppm for 3 months. The mean intake of MCPA was 0, 3.9, 11.8 and 35.4 mg/kg bw per day for males and 0, 4.4, 13.1 and 38.7 mg/kg bw per day for females at 0, 50, 150 and 450 ppm, respectively. Blood and urine were sampled from 10 rats of each sex per group on days 43 and 85 for the analysis of haematology, clinical chemistry or urine analysis parameters. At the end of treatment, all rats were assessed for gross pathological abnormalities, organs were weighed and histopathology was performed.

There were no deaths or clinical signs and no treatment-related effects on body weight gain or feed consumption. Ophthalmoscopy was unremarkable. Treatment-related effects occurred mainly in high-dose males, consisting of increased absolute ($P < 0.01$) and relative kidney weights (both about 14% higher than the control values), reduced serum calcium (day 43: 2.66 mmol/l versus 2.79 mmol/l in the controls, $P < 0.01$; day 85: 2.58 mmol/l versus 2.66 mmol/l in the controls, $P < 0.05$) and cholesterol (1.11 mmol/l versus 1.52 mmol/l in the controls at day 85, $P < 0.01$). In high-dose females, there was a significant increase in creatinine at day 85 (59.44 μmol/l versus 52.21 μmol/l in the controls; $P < 0.01$). There were no treatment-related macroscopic or histopathological abnormalities. The significantly increased ($P < 0.05$) absolute kidney weight in 150 ppm males was not considered treatment related, as it was not corroborated by a higher relative kidney weight, any histopathology or evidence of impaired renal function.

The NOAEL was 150 ppm (equal to 11.8 mg/kg bw per day), based on increased kidney weights, increased serum creatinine and reduced plasma calcium and cholesterol at the next higher dose of 450 ppm (equal to 35.4 mg/kg bw per day) (Kirsch et al., 1985b).

In a published study, Strauss et al. (2009) examined the influence of rat strain on the profile of endogenous metabolites (e.g. carbohydrates, amino acids) in blood following 28 days of dietary exposure to MCPA (unspecified purity) at a nominal concentration of 2500 ppm (five rats of each sex per strain). Control rats had access to untreated diet. The four rat strains examined were Crl:WI(Han), Han:RCC:WIST(SPF), Crl:CD® and F-344/Crl; the achieved doses were approximately 144, 151, 175 and 138 mg/kg bw per day for males and 169, 187, 189 and 137 mg/kg bw per day for females, respectively. Blood was sampled on days 7, 14 and 28 for the analysis of limited haematology and clinical chemistry parameters and endogenous metabolites.

There were no marked differences in endogenous metabolite profiles across the four strains following dietary exposure to MCPA. These profiles were consistent with reference patterns for peroxisome proliferation or renal organic anionic transporter inhibition. The adverse effects of MCPA were discernible across all four rat strains and included increased urea and creatinine (renal effects) and liver changes consistent with enzyme induction (decreased bilirubin and increased triglyceride) and slight hepatocyte membrane damage (increased ALT activity).

Dogs

In a 4-week range-finding study that predated principles of GLP, MCPA (purity 98.4%) was administered to one Beagle dog of each sex per group in the diet or in capsules (soya bean oil vehicle) at a nominal dose of 0, 8, 20 or 32 mg/kg bw per day. Standard gross toxicological end-points were recorded, with blood and urine sampled on days 25–27 for the analysis of haematology, clinical chemistry or urine analysis parameters. At the end of the study, survivors were killed and necropsied; organs were weighed and examined histopathologically.

Data were combined for each dose group, as there were no apparent sex differences. There were no deaths and no treatment-related clinical signs or ophthalmic abnormalities. Reduced body weight gain occurred in males at and above 20 mg/kg bw per day, whereas one high-dose female failed to gain body weight. Haematology and urine analysis parameters were unremarkable, whereas there was a dose-related increase in mean blood urea nitrogen and ALT (combined values for males and females: 10.1, 16.2, 17.7 and 19.6 mg% and 9.5, 15.9, 31.9 and 34.0 Reiman-Frankel units at 0, 8, 20 and 32 mg/kg bw per day, respectively). At the highest dose, mean absolute and relative thymus weights were about half those of the control group, with slight cortical atrophy of the thymus observed in one male and one female (Reuzel et al., 1978).

In a non-guideline study that predated principles of GLP, MCPA (purity 94.6%) was admixed in the diet and fed to groups of four Beagle dogs of each sex per group at a nominal dose of 0, 3, 12 or 48 mg/kg bw per day for 13 weeks. Satellite groups of four dogs of each sex per group were offered diets containing MCPA technical (purity 94.6%) at a nominal dose of 0, 0.3, 1 or 12 mg/kg bw per day or purified MCPA (purity 99.25%) at a single dose of 12 mg/kg bw per day. Dogs were examined daily for clinical signs. Ophthalmoscopy was performed prior to the commencement of dosing and during weeks 7 and 13. Body weight and feed consumption were recorded at various times throughout the study. Blood and urine were sampled pretreatment and during weeks 7 and 12 for the analysis of haematology and clinical chemistry or urine analysis parameters. In the satellite study, haematology was performed only on dogs in the 12 mg/kg bw per day groups. Faeces were analysed during weeks 1, 4, 8 and 12 for occult blood. Liver and kidney function tests were performed during week 13 using the bromosulfophthalein and phenolsulfonphthalein methods, respectively (no liver function test was performed in the satellite study). At the end of the main and satellite studies, survivors were sacrificed, gross pathological abnormalities were recorded, organs were weighed and histopathology was performed.

At 48 mg/kg bw per day, clinical signs occurred in seven of the eight dogs and included pustules, papules, necrotic skin lesions, focal stomatitis, conjunctivitis, diarrhoea, anorexia, dehydration, lethargy and signs of icterus. In addition, jaundice, bloody diarrhoea or urine, and bile vomiting were noted in two or three dogs. One of the seven dogs was found dead on day 48, with the remaining six sacrificed in a moribund condition on days 56–63. The remaining male dog, which exhibited no treatment-related clinical signs, was withdrawn from treatment during week 7. There were no deaths or treatment-related clinical signs at any of the lower doses, including in the satellite groups.

Ophthalmoscopy revealed slight conjunctivitis progressing to muco-purulent conjunctivitis in three males and three females at 48 mg/kg bw per day, with the three males also displaying corneal damage possibly resulting from the conjunctivitis. Two males and two females had jaundice of the schlera. No treatment-related ophthalmic abnormalities occurred at lower doses. Slight to moderate muco-purulent conjunctivitis was detected in the 12 mg/kg bw per day satellite group (one dog dosed with the MCPA technical and two dogs receiving purified MCPA).

At 48 mg/kg bw per day, the seven dogs that exhibited overt signs of toxicity failed to gain body weight, whereas reduced body weight gain was noted in the survivor. Reduced body weight gain occurred at 12 mg/kg bw per day in the main study (approximately 20% compared with the control group), but not in the satellite group, where body weight gain was unremarkable. At 48 mg/kg bw per

day, feed consumption was reduced during week 5 to the point where supplementary feeding with basal diet was necessary for 1 week. When feeding with the test diet was resumed, dogs ceased eating.

In the main study, perturbations in haematology parameters were observed most obviously in blood collected during week 6, with limited opportunity to analyse week 12 samples because of the high number of deaths at 48 mg/kg bw per day. At 48 mg/kg bw per day, reduced haemoglobin (6.6 mmol/l versus 8.6 mmol/l in the controls, $P < 0.05$), haematocrit (0.34 l/l versus 0.42 1/1 in the controls), erythrocytes (4.3×10^{12}/l versus 5.8×10^{12}/l in the controls) and thrombocytes (278×10^{9}/l versus 327×10^{9}/l in the controls) occurred in females during week 6. Similar evidence of anaemia was not observed at lower doses. In the main study, serum urea and creatinine were significantly elevated ($P < 0.05$) at and above 3 mg/kg bw per day during week 6 (approximately 1.4- to 2.8-fold and 1.2- to 1.4-fold higher than the control values, respectively). This was corroborated by significantly elevated ($P < 0.05$) urea and creatinine in the 12 mg/kg bw per day satellite groups during weeks 6 and 12 (up to about 1.5- and 1.3-fold higher than the control values, respectively). At 48 mg/kg bw per day, liver enzymes were elevated most obviously during week 6 because of the high number of deaths by week 12; relative to the control, AST was increased 3.7-fold in females, ALT by 2.9- or 11.7-fold (males and females, respectively) and ornithine transcarbamoylase by 40-fold in females. Additionally at 48 mg/kg bw per day, elevated bilirubin (53-fold in females) and an approximately 20% decrease in glucose ($P < 0.05$ in both sexes) were noted. In the satellite study, ornithine transcarbamoylase was elevated at 12 mg/kg bw per day (up to about 1.5- to 2.5-fold higher than the control value). Treatment-related urine analysis findings were confined to the presence of bilirubin (one male, three females) and increased epithelial cells (two females) at 48 mg/kg bw per day.

There was a significant ($P < 0.05$) dose-related increase in the concentration of urinary phenolsulfonphthalein at and above 3 mg/kg bw per day in the main study and at 12 mg/kg bw per day in the satellite study, which indicated that kidney function was adversely affected by treatment. Liver function was impaired in the surviving dog at 48 mg/kg bw per day. Faecal blood was detected in several dogs at 48 mg/kg bw per day and in two of the dogs fed purified MCPA at 12 mg/kg bw per day.

At 48 mg/kg bw per day, the relative kidney weights of dogs that were sacrificed in extremis were higher than those of the controls (approximately 20% in males). In the satellite groups, absolute and relative prostate weights were significantly lower ($P < 0.05$) than those of the control group at 12 mg/kg bw per day with technical MCPA (absolute: 1.72 g versus 4.37 g in the controls; relative: 0.15 versus 0.38 in the controls). However, given the large variation in prostate weights and the absence of similar findings with purified MCPA at the equivalent dose, these significant differences were not considered treatment related. In the main study, treatment-related macroscopic abnormalities included jaundice (three males and three females at 48 mg/kg bw per day), distended gall bladder (two males and two females at 12 mg/kg bw per day; all dogs at 48 mg/kg bw per day), gastrointestinal haemorrhages (two males and two females at 48 mg/kg bw per day) and pale or yellow liver (two males and two females at 48 mg/kg bw per day). No treatment-related macroscopic abnormalities were observed at lower doses or in the satellite groups. Histopathology revealed degenerative and/or regenerative effects in the liver (necrosis, inflammatory infiltrates, cytomegaly and karyomegaly, centrilobular liver cell degeneration, bile duct proliferation and the accumulation of brown bile pigment), kidneys (regeneration of the tubular epithelium in the intercorticomedullary layer) and gastrointestinal tract (mucosal haemorrhage, focal necrosis and polymorphonuclear cell infiltrates, cystic dilatation of the intestinal crypts) of dogs fed 48 mg/kg bw per day. There were no treatment-related microscopic abnormalities at the lower doses tested in the main study. In the satellite study at a dose of 12 mg/kg bw per day, slight to moderate bile duct proliferation was noted in some dogs fed technical MCPA (three males) or purified MCPA (two males).

The NOAEL was 1 mg/kg bw per day, based on increased serum creatinine and urea and reduced kidney function at 3 mg/kg bw per day (Reuzel et al., 1980).

In a published study conducted according to OECD Test Guideline No. 409, groups of four Beagle dogs of each sex per group received MCPA (unspecified purity) in gelatine capsules at a dose of 0, 1, 5 or 15 mg/kg bw per day for 90 days. An additional four dogs of each sex were allocated to the control and high-dose groups to study reversibility after 4 weeks of recovery. Blood was sampled at 30, 60 and 90 days for the analysis of clinical chemistry parameters. At necropsy, an extensive selection of tissues was weighed, and histological examinations were conducted.

There were no deaths, treatment-related clinical signs, ophthalmic abnormalities or effects on body weight or haematology parameters. Selected clinical chemistry parameters are presented in Table 13. Relative to the control group and pretreatment values, AST was significantly increased ($P < 0.05$) at and above 5 mg/kg bw per day in males and at 15 mg/kg bw per day in females. Serum urea was significantly elevated ($P < 0.05$) at 5 and 15 mg/kg bw per day in both sexes, whereas serum creatinine was significantly elevated ($P < 0.05$) at every dose in both sexes (noting that there was a significant [$P < 0.05$] time-related increase in serum creatinine in the controls). All of these clinical chemistry parameters recovered 28 days after the cessation of treatment. Mean testes weights were significantly decreased ($P < 0.05$) in high-dose males (right testis: 0.78 g versus 1.06 g in the controls; left testis: 0.73 g versus 1.05 g in the controls), but not in dogs allowed to recover for 4 weeks. Testicular atrophy was observed in one high-dose male, with a low occurrence of multinucleate giant cells, and a second male of this group showed an absence of spermatogenic cells in several tubules. Focal hepatocellular necrosis together with inflammatory change and mononuclear cell nodules was reported in treated animals, predominantly in high-dose males, but the incidence of this finding was unreported.

Table 13. Clinical chemistry findings in dogs following 90 days of capsular administration of MCPA

Parameter	Mean value							
	0 mg/kg bw per day		1 mg/kg bw per day		5 mg/kg bw per day		15 mg/kg bw per day	
	Males	Females	Males	Females	Males	Females	Males	Females
ALT (µkat/l)								
Pretreatment	0.59	0.84	0.60	0.62	0.71	0.79	0.78	0.81
Day 30	0.67	1.04	0.60	1.15	1.11*	0.94	1.37#	1.76+
Day 60	0.52	0.65	0.42	0.78	0.73	0.72	0.72	1.33#
Day 90	0.51	0.66	0.48	0.78	0.71	0.65	1.06#	1.28+
Recovery	0.53	0.73	—	—	—	—	0.48	0.61+
Urea (mmol/l)								
Pretreatment	3.34	4.27	3.60	5.04	3.70	3.91	4.32	4.53
Day 30	3.49	4.83	4.63	5.25	6.89#	6.38	7.35#	7.30#
Day 60	3.60	4.17	3.70	5.25	6.69	6.70*	7.10#	7.05#
Day 90	3.34	4.06	4.42	4.53	5.76*	6.38*	5.81*	6.43#
Recovery	3.67	4.43	—	—	—	—	3.24	5.51
Creatinine (µmol/l)								
Pretreatment	58.7	66.8	60.3	65.7	66.5	57.9	63.08	67.6
Day 30	61.0	81.8	66.5	99.0	79.6*	104.8#	81.1#	125.2#
Day 60	64.7	81.8+	71.2#	99.0#	90.9#	104.8*	96.6#	125.2#
Day 90	70.0	75.8+	77.6+	84.2#	99.4#	88.5#	94.0#	98.3#
Recovery	70.2	79.0+	—	—	—	—	67.3	78.3

From Sadloňová, Hózová & Flaškárová (2006)

— not analysed; * $P < 0.05$ compared with the control group; # $P < 0.05$ compared with the control and pretreatment value; + $P < 0.05$ compared with the pretreatment value

No NOAEL was identified. The lowest-observed-adverse-effect level (LOAEL) was 1 mg/kg bw per day, the lowest dose tested, based on elevated serum creatinine (Sadloňová, Hózová & Flaškárová, 2006).

MCPA 2-EHE (purity 93.5%) was admixed in the diet at a concentration of 0, 20, 80 or 360 ppm and fed to groups of four Beagle dogs of each sex per group for 110–118 days. Mean achieved doses of MCPA 2-EHE were, respectively, 0, 0.6, 2.5 and 11.1 mg/kg bw per day for males and 0, 0.7, 2.8 and 12.7 mg/kg bw per day for females (equal to 0, 0.4, 1.6 and 7.1 mg/kg bw per day for males and 0, 0.4, 1.6 and 8.1 mg/kg bw per day for females, respectively, expressed as acid equivalents). The study was conducted concurrently with and shared the control group for an identical study with MCPA DMA, reported below. Dogs were examined daily for clinical signs. Body weight was recorded weekly, and feed consumption daily. Ophthalmoscopy was performed pretreatment and at the end of the study (day 105 or 106). Blood and urine were collected pretreatment and during weeks 6 and 14 for the analysis of standard haematology, clinical chemistry or urine analysis parameters. At the end of the study, all dogs were assessed for gross pathological abnormalities, organs were weighed and histology was performed.

There were no deaths and no treatment-related clinical signs, ophthalmic abnormalities or effects on body weight or feed consumption. Haematology and urine analysis parameters were generally unremarkable, with the exception of partial thromboplastin time (PTT), which was significantly increased ($P < 0.05$) at the highest dose (Table 14). Significantly increased urea ($P < 0.01$; mid- and high-dose females) and creatinine ($P < 0.01$; mid- and high-dose males and females) were noted (Table 14). The significant increase ($P < 0.05$) in creatinine in low-dose males was considered an incidental finding, as there was no effect at the next higher dose (i.e. mid-dose). At the highest dose only, elevated ALT, serum magnesium and cholesterol (females only) and decreased blood glucose and ALP (females only) were noted (Table 14). There were no treatment-related macroscopic abnormalities or effects on organ weights. Histopathology revealed chronic interstitial hepatitis in four males and three females at the highest dose.

The NOAEL was 20 ppm (approximately equal to 0.4 mg/kg bw per day expressed as acid equivalents), based on increased serum urea and creatinine at the next higher dose of 80 ppm (approximately equal to 1.6 mg/kg bw per day expressed as acid equivalents) (Hellwig et al., 1995a).

In a study conducted concurrently and consistently with the preceding study, MCPA DMA salt (purity 99.9%; 63.4% acid equivalents) was admixed in the diet at a concentration of 0, 20, 80 or 360 ppm and fed to four Beagle dogs of each sex per group for 110–118 days. The achieved doses of MCPA DMA were, respectively, 0, 0.6, 2.4 and 10.9 mg/kg bw per day for males and 0, 0.7, 2.9 and 12.8 mg/kg bw per day for females. These doses are equal to approximately 0, 0.5, 2 and 9 mg/kg bw per day for males and approximately 0, 0.6, 2.4 and 10.5 mg/kg bw per day for females expressed as acid equivalents.

There were no deaths and no treatment-related clinical signs, ophthalmic abnormalities or effects on body weight or feed consumption. Consistent with the preceding study was the significant increase in PTT (high-dose females), ALT (high-dose males), serum creatinine (mid- and high-dose dogs of both sexes) and urea (high-dose dogs of both sexes, mid-dose females) and decreased ALP (high-dose males) (Table 15). There was no treatment-related effect on mean absolute or relative organ weights, and there were no treatment-related gross abnormalities. Subacute to chronic interstitial hepatitis was detected in one male and three females of the high-dose group. There were no microscopic findings at 20 or 80 ppm.

The NOAEL was 20 ppm (approximately equal to 0.5 and 0.6 mg/kg bw per day for males and females, respectively, expressed as acid equivalents), based on increased serum creatinine and urea at the next higher dietary concentration of 80 ppm (approximately equal to 2 and 2.4 mg/kg bw per day for males and females, respectively, expressed as acid equivalents) (Hellwig et al., 1995b).

Table 14. Clinical pathology findings in dogs exposed to MCPA 2-EHE in the diet

Parameter	Mean value							
	0 ppm		20 ppm		80 ppm		360 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
PTT (s)								
Pretreatment	11.3	11.8	11.4	11.2	11.6	12.0	11.4	11.7
Day 40 or 41	12.0	12.2	12.3	12.1	12.8	13.3	13.5	14.0*
Day 103 or 104	11.8	11.6	12.1	11.9	12.2	12.0	13.7*	12.9
ALT (µkat/l)								
Pretreatment	1.00	0.61	0.79	0.72	0.69	0.77	0.44*	0.64
Day 40 or 41	0.83	0.77	1.01	0.81	0.77	1.07	3.40	2.49
Day 103 or 104	0.81	0.72	0.87	0.8	0.80	0.93	3.05	1.71
ALP (µkat/l)								
Pretreatment	3.66	3.57	3.53	4.15	4.29	3.92	4.50	4.03
Day 40 or 41	2.61	3.21	2.83	3.00	2.43	2.74	2.56	1.93*
Day 103 or 104	2.56	2.68	2.57	3.03	2.12	2.61	2.12	1.79
Urea (µmol/l)								
Pretreatment	4.98	4.06	4.47	4.44	4.10	4.03	4.36	3.85
Day 40 or 41	4.28	5.05	4.89	5.61	5.14	5.35	6.18	7.55
Day 103 or 104	5.38	5.32	5.55	6.06	6.43	6.46*	7.55	7.3*
Creatinine (µmol/l)								
Pretreatment	75.3	69.4	79.1	69.9	70.3	69.5	69.0	66.5
Day 40 or 41	76.6	75.4	92.1*	77.1	86.3	84.6*	94.7*	97.9*
Day 103 or 104	80.8	80.0	87.9	85.8	95.1*	93.0	105.2*	102.7*
Glucose (µmol/l)								
Pretreatment	6.35	6.19	6.43	6.43	6.00	6.90	6.33	6.37
Day 40 or 41	6.17	6.47	6.57	6.19	6.18	6.49	5.98	6.22
Day 103 or 104	6.23	6.65	6.43	6.53	6.14	6.97	5.77*	5.89
Cholesterol (mmol/l)								
Pretreatment	6.05	5.10	5.02	4.44	4.54	4.61	5.04	5.05
Day 40 or 41	5.10	4.99	4.46	4.06	4.59	4.47	5.99	6.11
Day 103 or 104	5.47	4.66	4.57	4.10	4.67	6.51	5.44	7.30*
Magnesium (mmol/l)								
Pretreatment	0.71	0.82	0.79	0.81	0.71	0.82	0.78	0.83
Day 40 or 41	0.82	0.85	0.80	0.76	0.82	0.83	0.93	0.92
Day 103 or 104	0.68	0.85	0.67	0.82	0.75	0.88	0.88*	0.94*

From Hellwig et al. (1995a)
* $P < 0.05$

MCPA (purity 94.8%) was admixed in the diet at a concentration of 0, 6, 30 or 150 ppm and fed to six Beagle dogs of each sex per group for 52 weeks. Mean intakes of MCPA over 52 weeks were 0, 0.22, 1.1 and 5.7 mg/kg bw per day for males and 0, 0.22, 1.1 and 5.5 mg/kg bw per day for females at 0, 6, 30 and 150 ppm, respectively. Clinical observations were made, and feed consumption was recorded daily. Body weight was recorded weekly. Haematology, clinical chemistry and urine analysis parameters were analysed in blood or urine collected pretreatment and at weeks 13, 26 and 52. Ophthalmoscopy was performed pretreatment and at 6 months and 1 year. At the end of

the study, all survivors were killed and necropsied; organs were weighed and examined histopathologically. There were no deaths, treatment-related clinical signs or ophthalmic abnormalities. At 150 ppm, mean absolute body weight and body weight gain of males were lower than the control values over most of the study, reaching statistical significance ($P < 0.05$) from day 126 to day 161 and from day 42 to day 175, respectively; overall, these dogs gained about 1 kg less than the other groups. Body weight gain also tended to be lower in males at 30 ppm, but the decrease did not achieve statistical significance. There was no treatment-related effect on body weight or body weight gain in females. Two females at both 30 and 150 ppm had reduced feed consumption, with the mean feed intake of both groups up to about 10% lower than the control values from week 8.

Table 15. Clinical pathology findings in dogs exposed to MCPA DMA in the diet

Parameter	Mean value							
	0 ppm		20 ppm		80 ppm		360 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
PTT (s)								
Pretreatment	11.3	11.8	11.7	11.2	11.7	11.9	10.9	12.1
Day 40 or 41	12.0	12.2	12.1	12.0	12.8	12.8	12.9	14.6*
Day 103 or 104	11.8	11.6	12.1	11.4	13.1	12.6	12.7	13.1*
ALT (µkat/l)								
Pretreatment	1.00	0.61	0.60	0.86	0.65	1.00	1.00	0.51
Day 40 or 41	0.83	0.77	0.69	0.82	0.78	0.84	2.44*	1.39
Day 103 or 104	0.81	0.72	0.75	0.82	0.75	1.09	2.33*	1.28
ALP (µkat/l)								
Pretreatment	3.66	3.57	3.95	3.91	4.50	2.95	4.38	3.62
Day 40 or 41	2.61	3.21	2.98	3.21	3.12	2.91	2.13	1.98
Day 103 or 104	2.56	2.69	2.53	3.03	2.54	2.63	1.69*	1.87
Urea (µmol/l)								
Pretreatment	4.98	4.06	4.98	4.23	4.05	4.26	4.40	3.87
Day 40 or 41	4.28	6.05	4.53	6.03	4.28	6.70	6.67*	7.38
Day 103 or 104	5.38	5.32	5.68	6.14	5.48	6.75*	6.64	7.53*
Creatinine (µmol/l)								
Pretreatment	75.3	69.4	75.3	70.3	71.5	72.8	73.7	75.3
Day 40 or 41	76.6	75.4	82.9	80.4	88.8*	89.6	102.3*	102.9*
Day 103 or 104	80.8	80.0	85.9	83.7	93.0	100.6*	104.4*	106.9*

From Hellwig et al. (1995b)
* $P < 0.05$

There was no treatment-related effect on any haematology or urine analysis parameter. Selected clinical chemistry parameters are presented in Table 16. Plasma bilirubin was significantly elevated ($P < 0.01$ or 0.05) in males at 30 ppm (week 13) and 150 ppm (weeks 13 and 26). Serum creatinine was significantly elevated at 30 and 150 ppm in both sexes (weeks 13, 26 and 52). At 150 ppm, serum urea was significantly elevated in both sexes (weeks 13 and 26 in males; weeks 13, 26 and 52 in females). At 30 ppm, serum urea was also significantly elevated at some time points (week 13 in males; weeks 13, 26 and 52 in females). On occasion, serum creatinine and urea were significantly lower ($P < 0.05$) than the control values at 6 ppm, but this was discordant with the significant and consistent elevation in these parameters at the middle and high doses. In females, significantly elevated plasma potassium occurred at 30 ppm (week 13) and 150 ppm (weeks 26 and 52).

Table 16. Clinical chemistry parameters in dogs exposed to MCPA for up to 52 weeks in the diet

Parameter	Mean value							
	0 ppm		6 ppm		30 ppm		150 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
Bilirubin (µmol/l)								
Week 0	3.17	2.89	2.14	2.98	2.58	2.23	2.26	2.67
Week 13	2.60	3.42	3.14	3.70	3.68**	3.55	3.28*	3.88
Week 26	3.57	3.71	3.22	3.63	3.39	3.60	4.09**	5.21
Week 52	1.97	2.96	1.90	3.02	2.00	2.89	2.35	3.73
Creatinine (µmol/l)								
Week 0	67.19	71.91	71.16	71.40	77.03	75.82	76.38	68.18
Week 13	78.72	81.65	79.74	80.31	91.19*	91.58**	103.98**	96.45**
Week 26	84.40	77.29	79.49	78.77	94.42*	87.15**	112.14**	94.03**
Week 52	86.17	81.37	78.62*	86.51	93.18*	92.04**	107.61**	102.95**
Urea (mmol/l)								
Week 0	4.83	4.16	3.91	4.19	5.28	3.74	5.00	4.04
Week 13	4.95	6.36	5.28	5.95*	6.18*	6.60**	7.39**	7.40**
Week 26	6.12	5.03	5.52*	4.67	6.66	4.83**	7.80**	5.72**
Week 52	4.64	4.69	4.36	4.89	5.14	5.49**	5.83	5.15**
Potassium (mmol/l)								
Week 0	4.45	3.99	4.32	4.17	4.29	3.93	4.26	4.11
Week 13	4.19	4.11	4.26	4.18	4.43	4.35*	4.33	4.30
Week 26	3.94	3.84	4.12	4.05	4.20	4.04	4.19	4.17*
Week 52	4.12	3.88	3.99	4.20	4.10	4.16	4.18	4.23**

From Hellwig et al. (1986)
* $P < 0.05$; ** $P < 0.01$

The mean absolute and relative thyroid weights of 150 ppm males were significantly higher ($P < 0.05$) than the control group values (absolute: 0.94 g versus 0.67 g in the controls; relative: 0.0074 versus 0.006 in the controls). Gross pathology revealed whitish-yellowish dots in the thyroid parenchyma of two males from this group, with focal hyperplasia of the thyroid follicles observed in one of these animals.

At necropsy, a dark brown coloration of the kidneys was detected in four females of the 30 ppm group, all females of the 150 ppm group and four males of the 150 ppm group. Histopathology revealed pigment storage (probably lipofuscin, based on an examination of unstained sections, which revealed a yellowish-brown pigment) in the epithelia of the proximal portions of the renal tubules of all dogs. At 30 and 150 ppm, dogs appeared to have stored more pigment in comparison with the controls (Table 17).

The NOAEL was 6 ppm (equal to 0.22 mg/kg bw per day), based on elevated serum creatinine and urea and increased pigment storage in the proximal portions of the renal tubules at and above 30 ppm (equal to 1.1 mg/kg bw per day) (Hellwig et al., 1986).

Table 17. Incidence of pigment storage in the proximal portions of the renal tubules of dogs exposed to MCPA for 52 weeks

Degree of pigment storage	Incidence ($n = 6$)							
	0 ppm		6 ppm		30 ppm		150 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
Minimal	2	4	2	1	0	0	0	0
Slight	2	1	2	4	3	2	0	0
Moderate	2	1	2	1	3	4	1	4
Pronounced	0	0	0	0	0	0	3	0

From Hellwig et al. (1986)

(b) Dermal application

Rats

In a dermal toxicity study, MCPA 2-EHE (purity 93.5%) in olive oil was applied to the dorsal skin (10% of the body surface) of groups of five rats (Chbb=THOM (SPF) strain) of each sex under semi-occluded conditions at a dose of 0, 16, 160 or 1000 mg/kg bw per day for 6 hours/day, 5 days/week, for 4 weeks. The application site was examined daily for signs of irritation. Feed consumption and body weight were recorded weekly. Blood was sampled at the end of the study for the analysis of haematology and clinical chemistry parameters. Following sacrifice, all rats were necropsied, organs were weighed and tissues were histopathologically examined.

Treatment-related findings were confined to high-dose females, consisting of significantly reduced ($P < 0.01$) erythrocyte counts, haemoglobin and haematocrit, indicative of mild anaemia (7.38×10^{12}/l versus 7.85×10^{12}/l for the control, 8.8 mmol/l versus 9.5 mmol/l for the control and 0.4 l/l versus 0.43 l/l for the control, respectively). The NOAEL was therefore 160 mg/kg bw per day, based on reduced red cell parameters in females at 1000 mg/kg bw per day (Kirsch et al., 1995a).

The dermal toxicity of MCPA DMA (purity 91.7%) in distilled water was assessed under similar conditions to those described in Kirsch et al. (1995a) at a dose of 0, 12, 120 or 1000 mg/kg bw per day.

There were no findings suggestive of systemic toxicity, and therefore the NOAEL was 1000 mg/kg bw per day, the highest dose tested. All high-dose rats had skin reactions at the application site throughout most of the exposure period consisting of slight to well-defined erythema, scaling and superficial scabbing. Minimal to moderate acanthosis (three males, four females) and slight hyperkeratosis (one male, two females) were observed histologically at the highest dose (Kirsch et al., 1995b).

Rabbits

The dermal toxicity of MCPA (purity 94.22%) in distilled water was assessed in groups of five New Zealand White rabbits of each sex per dose at a dose of 0, 10, 100 or 1000 mg/kg bw per day. The test substance was applied to the dorsal skin (approximately 10% of the body surface) under semi-occluded conditions for 6 hours/day, 5 days/week, for 4 weeks. Dermal responses were recorded before the first application and thereafter on a daily basis. Blood and urine were sampled at the end of the study for the analysis of haematology, clinical chemistry or urine analysis parameters. Following sacrifice, all rabbits were necropsied, organs weighed and tissues examined histopathologically.

There were no findings suggestive of systemic toxicity, and therefore the NOAEL was 1000 mg/kg bw per day, the highest dose tested. Skin reactions at the application site were observed at 100 and 1000 mg/kg bw per day. At 100 mg/kg bw per day, slight erythema and oedema were noted, with minimal diffuse acanthosis also observed in some animals. At 1000 mg/kg bw per day, erythema

and oedema progressing from slight to well defined were recorded in 5 of the 10 animals by day 10. Varying levels of discoloration, dryness and desquamation also occurred. Minimal diffuse acanthosis with or without hyperkeratosis of the treated skin was observed histologically in all high-dose animals (Baldrick et al., 1992).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

MCPA (purity 94.8%) was admixed in the diet at a concentration of 0, 20, 100 or 500 ppm and fed ad libitum to groups of male and female B6C3F1 mice for 104 weeks. The mean intakes of MCPA were 0, 3.4, 16 and 83 mg/kg bw per day for males and 0, 4.2, 21 and 103 mg/kg bw per day for females at 0, 20, 100 and 500 ppm, respectively. Each dose group consisted of one main cohort of 50 mice of each sex and a satellite group of 10 mice of each sex. Selection of the highest dose was based on a 4-month pilot study in which clinical chemistry changes suggestive of nephrotoxicity occurred at 900 ppm. Mice were observed daily for mortalities and clinical signs. Body weight and feed consumption were recorded weekly to 3 months and every 4 weeks thereafter. Blood was sampled at approximately 52 (satellite groups) and 104 weeks (10 mice from each of the main groups) for the analysis of haematology parameters; no clinical chemistry parameters were analysed. Following sacrifice (week 52 for the satellite groups and week 104 for the main groups), mice were examined for gross pathological abnormalities, and organs were weighed and examined histologically. There were no treatment-related mortalities, clinical signs or effects on body weight or feed consumption. It was noted that while the mean body weight of 500 ppm males from the main group was approximately 5% lower than the control value over most of the study (reaching statistical significance at some time points), there was no such difference over the same period of time in the satellite group or in females. On this basis, these small differences in body weight were not considered treatment related.

Haematology was generally unremarkable. In females at 500 ppm at 104 weeks, there was an increase in the number of mice with atypical lymphocytes (2/10, 3/10, 0/10 and 7/10 at 0, 20, 100 and 500 ppm, respectively) and Howell-Jolly bodies in erythrocytes (5/10 versus 2/9 in the controls); with no similar findings at 52 weeks or in males, the poor dose–response relationship and the “not very strongly pronounced” nature of the finding, this increase is unlikely to be treatment related.

In 100 and 500 ppm females, mean absolute and relative heart weights were approximately 8% lower than the control values ($P < 0.05$), but in the absence of a dose–response relationship or any histopathological abnormalities, these findings were not attributable to treatment. Mean absolute and relative kidney weights in 500 ppm females were approximately 10% higher ($P < 0.01$ or 0.05) than the control values. There were no treatment-related gross abnormalities. In both sexes at 500 ppm, there was an increased incidence of intratubular calcification (9/50, 11/50, 11/50 and 27/50 in males and 0/50, 1/50, 2/50 and 9/50 in females at 0, 50, 100 and 500 ppm, respectively) and hyaline casts (26/50, 32/50, 25/50 and 36/50 in males and 15/50, 14/50, 26/50 and 33/50 in females at 0, 50, 100 and 500 ppm, respectively). Tubular epithelial hyperplasia was increased in 500 ppm males (9/50, 12/50, 10/50 and 32/50 at 0, 20, 100 and 500 ppm, respectively).

There were no treatment-related neoplastic or non-neoplastic lesions. Under the conditions of the study, MCPA showed no carcinogenic potential in B6C3F1 mice.

The NOAEL was 100 ppm (equal to 16 mg/kg bw per day), based on nephrotoxicity (increased kidney weight, intratubular calcification, hyaline casts and tubular epithelial hyperplasia) at 500 ppm (equal to 83 mg/kg bw per day) (Kühborth et al., 1988).

Rats

MCPA (purity 94.8%) was admixed in the diet at a concentration of 0, 20, 80 or 320 ppm and fed ad libitum to groups of 50 Wistar rats of each sex for 24 months. Two satellite groups of 10 and 15 rats of each sex per dose (satellite groups I and II, respectively) were also included in the study. The mean achieved doses of MCPA (determined for the main cohort and satellite group I) were 0, 1.2,

4.8 and 19 mg/kg bw per day for males and 0, 1.5, 6.1 and 24.3 mg/kg bw per day for females at 0, 20, 80 and 320 ppm, respectively. Rats were observed daily for mortalities and clinical signs. Body weight and feed consumption were recorded weekly to week 14, then monthly thereafter. Ophthalmology was conducted at the beginning of the study and subsequently every 6 months in the control and high-dose groups from the main cohort. Blood was collected from the main cohort and satellite group II pretreatment and at about weeks 26, 52, 78 and 104 for the analysis of standard haematology and clinical chemistry parameters. Blood was collected during week 52 from satellite group I for the analysis of T_3 and thyroxine (T_4). Urine was collected from satellite group I at 26 and 52 weeks and from 10 rats of each sex per dose in the main cohort at 104 weeks for the analysis of urine analysis parameters. Survivors in satellite group I, in satellite group II and in the main cohort were killed after 12, 24 and 24 months, respectively. Gross pathological and histopathological examinations were performed on all rats. There were no treatment-related mortalities or clinical signs. At 320 ppm in the main cohort, the mean body weight of males was up to 9% lower than the control value, occasionally reaching statistical significance ($P < 0.05$). A similar difference in absolute body weight did not occur in the satellite groups over comparable time frames or in females at the same dose, where in fact significantly higher ($P < 0.05$) absolute body weights were determined at some time points. Feed consumption was unaffected by treatment. There were no treatment-related ophthalmic abnormalities.

Significant increases in plasma ALT activity showed no dose-, time- or sex-related consistency and were not corroborated by effects on other liver enzymes or histological effects consistent with liver toxicity (Table 18). In addition, the statistically significant increases were relatively modest (< 20%) and were not corroborated by the results of two subchronic studies. At 320 ppm, plasma triglycerides were significantly lower ($P < 0.01$ or 0.05) than the control values in males (weeks 78 and 104) and females (weeks 52 and 78). At 80 ppm, significantly lower ($P < 0.05$) triglycerides were determined in the blood collected from females during week 52 and males at week 104 (Table 18). These results might reasonably be expected, as MCPA is a known peroxisome proliferator. On this basis, the effects on triglycerides are considered to reflect a pharmacological rather than a toxicological effect.

Table 18. Selected clinical chemistry findings in rats exposed to MCPA in the diet for up to 2 years

Parameter	Mean value							
	0 ppm		20 ppm		80 ppm		320 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
ALT (µkat/l)								
Week 0	1.24	1.04	1.31	1.13	1.32	1.04	1.44**	1.06
Week 26	1.09	0.90	1.05	0.88	1.37	0.85	1.31*	0.94
Week 52	0.85	0.87	0.97**	0.91	1.05**	0.97**	0.95	1.04**
Week 78	0.81	0.81	0.84	0.88	0.95	0.86	0.87	0.93*
Week 104	0.81	0.79	0.79	0.80	0.84	0.80	0.91	0.92*
Triglycerides (mmol/l)								
Week 0	1.27	1.65	1.22	1.68	1.28	1.69	1.35	1.87
Week 26	3.35	4.62	3.92	3.31	4.55	3.81	3.39	3.82
Week 52	5.10	5.62	4.02	4.35	4.53	3.86*	3.58	3.38**
Week 78	5.60	6.55	4.21	5.78	5.19	5.45	3.81*	4.93*
Week 104	6.08	6.43	4.67	6.68	4.02*	6.11	3.94**	6.23

From Kirsch et al. (1988)
* $P < 0.05$; ** $P < 0.01$

There were no treatment-related macroscopic abnormalities. The mean absolute and relative kidney weights of 320 ppm males (satellite group I) were approximately 13% higher than the control

values at the 12-month sacrifice, but only the difference in absolute kidney weight was statistically significant ($P < 0.05$). There was no similar finding in females or in both sexes at 24 months. The increased kidney weight in 320 ppm males at 52 weeks was coincident with an increase in the severity of chronic-progressive nephropathy (Table 19). Also at the highest dose, only in rats sacrificed at 52 weeks, there was an increase in the severity of pigment storage in the spleen. However, this finding occurred without any haematological correlates or pigment storage in other tissues. The mean relative (but not absolute) liver weight of 320 ppm females was significantly lower ($P < 0.01$) than the control value at both the 12-month (satellite group I) and 24-month sacrifice (main cohort) (13% and 8% lower than the control values, respectively). In the absence of any treatment-related histopathological abnormalities of the liver, these lower weights are attributable to the lower terminal body weight of the group. There were no treatment-related neoplastic lesions.

Table 19. Grading of chronic progressive nephropathy and pigment storage in the spleen in rats sacrificed after 12 months

Finding	Incidence ($n = 10$)							
	Males				Females			
	0 ppm	20 ppm	80 ppm	320 ppm	0 ppm	20 ppm	80 ppm	320 ppm
Chronic progressive nephropathy								
Total	10	10	10	10	10	10	10	10
Slight	9	5	5	2	3	9	6	4
Minimal	1	5	5	2	1	1	2	1
Moderate	0	0	0	6	0	0	0	0
Pigment storage in the spleen								
Total	10	9	9	10	10	10	10	10
Slight	7	6	7	3	2	6	3	1
Minimal	3	3	2	5	8	4	6	6
Moderate	0	0	0	2	0	0	1	3

From Kirsch et al. (1988)

The NOAEL was 80 ppm (equal to 4.8 mg/kg bw per day), based on increased kidney weight and slight increases in the severity of chronic progressive nephropathy in males and haemosiderin deposition at 320 ppm (equal to 19 mg/kg bw per day). MCPA had no carcinogenic potential under the conditions of the study (Kirsch et al., 1988).

2.4 Genotoxicity

The results of genotoxicity studies on MCPA, MCPA 2-EHE and MCPA DMA are summarized in Tables 20, 21 and 22, respectively. All unpublished studies contained statements of compliance with principles of GLP or international/national test guidelines.

Table 20. Results of genotoxicity assays on MCPA

End-point	Test object	Concentration; vehicle	Purity (%)	Results	Reference
In vitro studies					
Gene mutation	*Salmonella typhimurium* TA98, TA100, TA1535 and TA1537	5–1000 µg/plate ±S9; ethanol vehicle	94.2	Negative	Jones et al. (1993a)[a]

End-point	Test object	Concentration; vehicle	Purity (%)	Results	Reference
Gene mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA1538	Up to 5000 µg/plate ±S9; DMSO vehicle	NR	Negative	Moriya et al. (1983)
Gene mutation	*S. typhimurium* TA97a, TA98, TA100, TA1535, TA1537 and TA1538	10–1000 µg/plate ±S9; ethanol or DMSO vehicle	NR	Negative[b]	Kappas (1988)
Gene mutation	*S. typhimurium* TA98 and TA100	1 µmol/plate ±S9; DMSO vehicle	NR	Negative	Nishimura et al. (1982)
Gene mutation	*Escherichia coli* WP2 (hcr)	Up to 5000 µg/plate ±S9; DMSO vehicle	NR	Negative	Moriya et al. (1983)
Gene mutation	*Saccharomyces cerevisiae*	Up to 6 µmol/l; ethanol vehicle	NR	Negative[c]	Zetterberg (1979)
Gene mutation	CHO cells	50–1200 µg/ml; ethanol vehicle	94.2	Negative[d]	Adams et al. (1993a)[a]
Gene mutation	Chinese hamster V79 cells	0.05–2 µg/ml; culture medium vehicle	NR	Negative	Fiskesjö (1988)
Chromosomal aberration	Human lymphocytes	50–2000 µg/ml ±S9	94.2	Negative −S9 Positive[e] +S9	Akhurst et al. (1993a)[a]
DNA damage and repair, SCE	CHO cells	10^{-5}, 10^{-4} and 10^{-3} mol/l ±S9; culture medium vehicle	NR	Negative	Linnainmaa (1984)
DNA damage and repair, SCE	Chick embryos	0.35–2.6 mg/embryo or 0.1–4.2 mg/embryo	95	Positive[f]	Arias (1992, 1996)
DNA damage and repair, mitotic segregation	*Aspergillus nidulans*	750–3000 µmol/l; ethanol or DMSO vehicle	NR	Positive −S9[g] Negative +S9	Kappas (1988)
In vivo studies					
Micronucleus	Swiss CD-1 mice, bone marrow	0, 96, 192 or 383 mg/kg bw; 1% CMC	94.2	Negative	Proudlock et al. (1993a)[a]
Cytogenicity	Chinese hamsters, bone marrow	0, 33, 200 or 1200 mg/kg bw; 0.5% CMC	94.8	Negative	Engelhardt & Gelbke (1985c, 1986)[a]
Sex-linked recessive	*Drosophila melanogaster*	5 mmol/l in the diet for 3 days	NR	Negative	Vogel & Chandler (1973)
SCE	Chinese hamsters, bone marrow	0, 33, 200 or 1200 mg/kg bw; 0.5% CMC	94.8	Negative	Engelhardt & Gelbke (1985a,b)[a]
SCE	Chinese hamsters and Wistar rats, bone marrow	100 mg/kg bw per day for 2 weeks by gavage; saline vehicle	NR	Negative	Linnainmaa (1984)

Table 20 (continued)

End-point	Test object	Concentration; vehicle	Purity (%)	Results	Reference
SCE	Wistar rats, lymphocytes	100 mg/kg bw per day for 2 weeks by gavage; DMSO vehicle	99	Negative	Mustonen et al. (1989)
DNA binding	SD male rats, liver	50 or 500 mg/kg bw; distilled water vehicle	97	Negative	McGregor (1986)

CHO, Chinese hamster ovary; CMC, carboxymethylcellulose; DMSO, dimethyl sulfoxide; DNA, deoxyribonucleic acid; NR, not reported; S9, 9000 × *g* supernatant fraction of rat liver homogenate; SCE, sister chromatid exchange; SD, Sprague-Dawley

[a] Conducted in compliance with GLP.

[b] Significant increase in revertants at 250 ($P < 0.05$), 500 ($P < 0.01$) and 750 ($P < 0.05$) µg/plate in the presence of S9 in strain TA97a, but all increases were less than 1.3-fold above the control; no increases observed in the absence of S9 or in any of the other tested strains.

[c] Approximately 1.3-fold increase in mutants compared with the control at highly cytotoxic concentrations, where cell survival was less than 5%.

[d] Cytotoxicity at 1000 µg/ml and above.

[e] Significant increase ($P < 0.001$) in aberrant cells at 1750 µg/ml and at 1250 and 1500 µg/ml in a repeat test in the presence of S9 only. These positive responses occurred only in the presence of cytotoxicity.

[f] There was a 1.3-fold increase ($P = 0.048$) in SCEs per cell at 2.8 mg/embryo; significant increase ($P < 0.05$; < 2-fold higher than the control) in SCEs per cell at 2.8 and 4.2 mg/embryo concomitant with mortality (21–42% at 1.4–4.2 mg/embryo).

[g] Up to a 2-fold, non-significant increase in mitotic segregation compared with the vehicle control at 2250 and 3000 µmol/l concomitant with toxicity of approximately 20–30%.

Table 21. Results of genotoxicity assays on MCPA 2-EHE

End-point	Test object	Concentration; vehicle	Purity (%)	Results	Reference[a]
In vitro studies					
Gene mutation	*Salmonella typhimurium* TA98, TA100, TA1535 and TA1537	50–5000 µg/plate ±S9; water vehicle	93.9	Negative	Jones et al. (1993b)
Gene mutation	CHO cells (HGPRT locus)	10–200 µg/plate ±S9; ethanol vehicle	93.9	Negative	Adams et al. (1993c)
Chromosomal aberration	Human lymphocytes	10–320 µg/ml ±S9; ethanol vehicle	93.9	Negative[b]	Akhurst et al. (1993c)
In vivo studies					
Micronucleus	Swiss CD-1 mice, bone marrow	0, 450, 900 or 1800 mg/kg bw in 1% CMC	93.9	Negative	Proudlock et al. (1993c)

CHO, Chinese hamster ovary; CMC, carboxymethylcellulose; S9, 9000 × *g* supernatant fraction of rat liver homogenate

[a] All studies conducted in compliance with GLP.

[b] Toxicity and cell cycle delay at and above 80 g/ml.

Table 22. Results of genotoxicity assays on MCPA DMA

End-point	Test object	Concentration; vehicle	Purity (%)	Results	Reference[a]
In vitro studies					
Gene mutation	*Salmonella typhimurium* TA98, TA100, TA1535 and TA1537	50–5000 µg/plate ±S9; water vehicle	77.3	Negative	Jones et al. (1992)
Gene mutation	CHO cells (HGPRT locus)	250–3000 µg/plate ±S9; water vehicle	77.3	Negative[b]	Adams et al. (1993b)
Chromosomal aberration	Human lymphocytes	100–5000 µg/ml ±S9; water vehicle	77.3	Negative −S9 Positive +S9[c]	Akhurst et al. (1993b)
In vivo studies					
Micronucleus	Swiss CD-1 mice, bone marrow	0, 144, 288 or 576 mg/kg bw; water vehicle	77.3	Negative[d]	Proudlock et al. (1993b)

CHO, Chinese hamster ovary; S9, 9000 × *g* supernatant fraction of rat liver homogenate

[a] All studies conducted in compliance with GLP.

[b] Cytotoxicity at 1250 µg/ml and above.

[c] Significant increase ($P < 0.05$) in aberrant cells (2.5% versus 0.5% in the controls) at 1000 µg/ml in the absence of S9; significant increases in aberrant cells at 250 ($P < 0.01$), 1000 ($P < 0.001$) and 2000 µg/ml ($P < 0.001$) (3.5%, 5% and 13.5% versus 0.5% in the controls, respectively); cytotoxicity at 2000 µg/ml and above.

[d] Decrease in polychromatic erythrocytes at 48 hours, indicative of a transient depression in bone marrow.

2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

Rats

MCPA (purity 94.8%) was admixed in the diet at a concentration of 0, 50, 150 or 450 ppm and fed ad libitum to two parental generations of Crl:CD®(SD)BR rats (25 of each sex per group) and their offspring. Rats were observed for mortalities and clinical signs at least twice daily, with a more detailed clinical assessment made weekly. Body weight and feed consumption were recorded prior to treatment and weekly during the premating period; dams were weighed on days 0, 4, 7, 14 and 20 of gestation. Standard reproduction and litter parameters were recorded or calculated. Necropsies were performed on all parental rats and 10 pups of each sex per group. The following tissues were examined histopathologically in all control and high-dose F_0 and F_{1b} adult rats: gross lesions, epididymides, kidneys, ovaries, prostate, seminal vesicles, testes, uterus and vagina.

Estimated doses received by rats throughout all phases of the study were calculated by the sponsor based on weekly feed consumption and mean body weight and are presented in Table 23.

There were no treatment-related deaths or clinical signs. Body weight gain was unremarkable in F_0 and F_1 parental rats, noting that absolute body weight was significantly lower ($P < 0.05$) than the control value (by about 10%) at 450 ppm in F_1 rats of both sexes early in the premating period. Feed consumption by F_0 parental rats was unremarkable, whereas significantly higher ($P < 0.05$) feed consumption was determined at 450 ppm in F_{1b} females during the premating and rest periods (up to 13% higher than the control value) and in F_{1b} males during the rest period (approximately 5% higher than the control value); these increases in feed consumption are not considered toxicologically significant.

Table 23. Doses of MCPA received by parental rats

Phase	Dose (mg/kg bw per day)					
	50 ppm		150 ppm		450 ppm	
	Males	Females	Males	Females	Males	Females
F_0 premating						
Week 1	5.3	5.5	15.7	17.5	47.4	52.0
Week 5	3.5	4.4	10.4	13.4	32.3	41.5
Week 10	2.8	3.5	8.3	11.4	25.2	32.4
Mean	3.6	4.4	10.7	12.7	39.6	41.0
F_1 premating						
Week 1	7.7	7.9	22.8	23.9	72.5	78.6
Week 5	4.0	5.0	12.2	14.4	37.1	47.4
Week 10	3.1	3.9	8.9	11.7	28.2	37.7
Mean	4.5	5.2	13.4	15.5	41.5	45.8

From MacKenzie (1986); Bellet et al. (2000)

At 450 ppm, body weight gain between days 14 and 21 for F_{1a} pups (males and females; approximately 12% lower than the control value) and F_{1b} pups (females; approximately 9% lower than the control value) was significantly lower ($P < 0.05$) than the control value. The absolute body weight of F_{2a} pups was significantly lower ($P < 0.05$) than the control value on day 14 (males; 8% lower) and day 21 (both sexes; approximately 10% lower). Body weight gain by F_{2a} male pups was significantly lower than the control value ($P < 0.05$) at 150 ppm (days 14–21, 9% lower) and 450 ppm (days 4–14, 9% lower; and days 14–21; 14% lower). In the F_{2b} litters, female body weight (day 4) and body weight gain (days 0–4) were significantly lower ($P < 0.05$; approximately 15% lower) than the control value at 50 ppm, but not at higher doses or other times. At 450 ppm, absolute body weight of F_{2b} pups at day 14 (males; approximately 7% lower) and day 21 (males and females; approximately 8% lower) and female body weight gain between days 14 and 21 (approximately 12%) were significantly lower ($P < 0.05$) than the control values. The consistency of the lower absolute body weight and body weight gain in pups at 450 ppm is clearly attributable to treatment. Significant differences in either absolute body weight or body weight gain at 50 or 150 ppm showed no consistency between generations, litters or sexes and on this basis are unlikely to be treatment related.

There was no treatment-related effect on reproduction or litter parameters or the incidence of gross or histopathological abnormalities.

The NOAEL for reproductive toxicity was 450 ppm (approximately equal to 40 mg/kg bw per day), the highest dose tested. The NOAEL for parental toxicity was 150 ppm (approximately equal to 12 mg/kg bw per day), based on lower absolute body weight at 450 ppm. The NOAEL for pup toxicity was also 150 ppm, based on reduced body weight gain at 450 ppm (MacKenzie, 1986; Bellet et al., 2000).

A one-generation reproduction study was conducted in groups of Alpk:AP$_f$SD (Wistar-derived) rats (12 of each sex per group) that were fed diets containing MCPA (purity 97.4%) at a concentration of 0, 450, 750 or 1000 ppm. As the purpose of the study was to clarify the dose–response relationship in rat pups compared with adults, feed consumption was measured throughout all phases of the study to allow an accurate determination of the achieved dose. From day 1 postpartum, the concentrations of MCPA in the diet were reduced to 300, 500 and 667 ppm, respectively, to avoid administering particularly high doses to lactating dams and pups. Litters were raised to weaning (day 29 postpartum), then 10 F_1 pups of each sex were selected for a further 2 weeks of exposure to the premating dietary concentrations (450, 750 and 1000 ppm, respectively). Body weight was recorded at least weekly. Reproductive performance was determined by examining

mating success, gestation length and precoital interval. Pup survival and body weight were recorded during lactation. Parental rats and offspring were necropsied. Kidney, liver and ovaries or testes were weighed in parental rats and selected F_1 offspring. Achieved doses throughout the study are presented in Table 24.

Table 24. Achieved doses of MCPA in rats

Stage	Achieved dose (mg/kg bw per day)					
	450 ppm		750 ppm		1000 ppm	
	Males	Females	Males	Females	Males	Females
F_0 premating	38.9	41.7	67.1	69.2	88.9	89.0
Gestation	—	35.7	—	57.0	—	75.4
Lactation	—	62.6	—	98.8	—	122.2
Selected F_1 rats	76.1	72.2	114.7	114.9	157.4	156.1

From Milburn (2004a)

There were no treatment-related deaths or clinical signs. During the premating exposure period at 1000 ppm, absolute body weight was significantly lower than the control value during most weeks ($P < 0.01$ or 0.05; up to 9% and 6% lower than the control values in males and females, respectively). At 750 and 450 ppm, the difference in absolute body weight was significant only within the first 3 weeks of treatment ($P < 0.05$; up to 4% lower than the control value). During gestation, the mean body weight of 1000 ppm dams was significantly lower than the control value on day 1 ($P < 0.05$; 5% lower than the control value). There was no treatment-related effect on body weight during lactation. These lower body weights were coincident with significantly lower ($P < 0.01$ or 0.05) feed consumption.

Pup body weights were significantly lower ($P < 0.01$; approximately 8% lower than the control value) only at the highest dietary concentration (667 ppm) on day 29 of lactation.

In selected F_1 rats, mean absolute body weights were significantly lower ($P < 0.01$ or 0.05) than the control value across all doses and in both sexes; the magnitude of the difference relative to the control group was very similar to that occurring during the premating period.

There was no treatment-related effect on reproduction or litter parameters.

There were no treatment-related macroscopic abnormalities.

Absolute liver, ovary and testes weights were significantly lower ($P < 0.01$ or 0.05) than the control values in high-dose F_1 males and females (approximately 10% lower), but when adjusted for body weight, there was no difference relative to the controls. Relative kidney weight was significantly elevated ($P < 0.05$; approximately 10% higher than the control value) in high-dose males.

The NOAEL for reproductive toxicity was 1000 ppm (approximately equal to 160 mg/kg bw per day), the highest dose tested. The NOAEL for parental toxicity was less than 450 ppm (approximately equal to 40 mg/kg bw per day), based on reduced body weight observed at this dose. The NOAEL for offspring toxicity was 750 ppm (approximately equal to 115 mg/kg bw per day), based on reduced body weight at the highest dose of 1000 ppm (approximately equal to 160 mg/kg bw per day) (Milburn, 2004a).

A one-generation reproduction study on MCPA 2-EHE (purity 96.2%) was conducted in parallel with the preceding study by Milburn (2004a) and shared a control group. The experimental conditions were comparable, except that the concentrations of test material in the diet were 0, 700, 1200 and 1600 ppm during the premating period and 2-week exposure period of F_1 rats, decreasing to

0, 467, 800 and 1067 ppm, respectively, during lactation. Achieved doses throughout the study are presented in Table 25.

Table 25. Achieved doses in rats

Stage	Achieved dose (mg/kg bw per day expressed as MCPA acid equivalents)					
	700 ppm		1200 ppm		1600 ppm	
	Male	Female	Male	Female	Male	Female
F_0 premating	64.6	66.3	105.9	112.8	144.9	151.2
Gestation	—	54.0	—	90.3	—	126.4
Lactation	—	91.6	—	162.9	—	217.1
Selected F_1 rats	107.1	106.7	178.4	187.9	232.3	249.1

From Milburn (2004b)

There were no treatment-related deaths or clinical signs. During the premating period (mainly weeks 2–5), the mean body weight of F_0 rats was significantly lower ($P < 0.01$ or 0.05) than the control value at 700 ppm (up to about 3–4% lower), 1200 ppm (up to about 5–6% lower) and 1600 ppm (up to about 5–7% lower). At 1200 and 1600 ppm, the body weight of dams was significantly lower ($P < 0.05$) than the control value on day 15 of gestation (4% and 6% lower, respectively). At 1600 ppm, the mean absolute body weights of F_1 males and females were up to 13% and 11% lower ($P < 0.01$ or 0.05) than the control values, respectively, during the 2-week post-selection exposure period. At 700 and 1200 ppm, body weight was maximally 3–4% and 7–8% lower than the control values, respectively, but was statistically significantly lower ($P < 0.01$) only for low-dose males at week 2. At 1600 ppm, significantly lower ($P < 0.05$) feed consumption was noted in F_0 females during week 1 of the premating period and F_1 males at 1200 ppm (week 2 of the post-selection period) and 1600 ppm (weeks 1 and 2 of the post-selection period).

There was no effect on reproductive performance, pup survival or litter size. At 1600/1067 ppm, male and female pup weights were 5% and 9% lower ($P < 0.05$ in males; $P < 0.01$ in females) than the control values, respectively, on day 29 of lactation. At 1200/800 ppm, female pup weights were 6% lower ($P < 0.05$) than the control weights on day 29 of lactation.

There were no treatment-related macroscopic abnormalities. In F_0 dams, absolute (but not relative) kidney weights were significantly higher ($P < 0.01$ or 0.05) than the control values across all doses, without a clear dose–response relationship (absolute: 2.21, 2.49, 2.46 and 2.48 g at 0, 700, 1200 and 1600 ppm, respectively; relative: 0.69%, 0.77%, 0.75% and 0.78% at 0, 700, 1200 and 1600 ppm, respectively). At 1600 ppm, the absolute weights of the liver and testes of F_1 males were significantly lower ($P < 0.01$ or 0.05) than the control values (liver: 9.8 g versus 11.3 g; testes: 1.80 g versus 1.96 g). Absolute ovary weights were also significantly lower ($P < 0.01$ or 0.05) than the control values across all doses (0.083, 0.069, 0.066 and 0.069 g at 0, 700, 1200 and 1600 ppm, respectively). These apparent reductions in kidney, ovary, liver and testes weights were considered to reflect the reduced body weight of immature rats.

The NOAEL for reproductive toxicity was 1600 ppm (approximately equal to 150 mg/kg bw per day expressed as acid equivalents), the highest dose tested. The NOAEL for parental toxicity was less than 700 ppm (approximately equal to 65 mg/kg bw per day expressed as acid equivalents), based on reduced body weight at this dose. The NOAEL for offspring toxicity was 1200 ppm (equal to a minimum of about 90 mg/kg bw per day expressed as acid equivalents), based on reduced body weight at 1600 ppm (approximately equal to 230 mg/kg bw per day expressed as acid equivalents) (Milburn, 2004b).

(b) Developmental toxicity

Rats

In a pre-GLP/guideline range-finding study, MCPA (unspecified purity) was administered in 1% w/v carboxymethylcellulose by gavage to groups of five mated SD rats (CD strain) at a dose of 25 or 100 mg/kg bw per day from day 0 to day 20 of gestation. Ten control rats received the vehicle only. Observations for clinical signs were made daily throughout the dosing period. Body weight was recorded every 3 days. All dams were sacrificed on day 21 of gestation; fetuses were examined for external, visceral and skeletal abnormalities.

There were no maternal deaths or treatment-related clinical signs. Body weight gain was approximately 22% and 13% lower than the control values at 25 and 100 mg/kg bw per day, respectively. Fetal weight and crown–rump length were significantly lower ($P < 0.05$) than the control values at 100 mg/kg bw per day (fetal weight: 4.9 g versus 5.9 g in the controls; crown–rump length: 40.8 mm versus 44.1 mm in the controls), but all values were stated to be within the normal range for this particular rat strain (Irvine & Tucker, 1978a).

In the follow-up study by Irvine (1980a), MCPA (unspecified purity) was administered by gavage to groups of five mated SD rats (CD strain) in 1% w/v carboxymethylcellulose at a dose of 0, 20, 50 or 125 mg/kg bw per day from day 6 to day 15 of gestation. Observations for clinical signs were made daily, whereas body weight and feed consumption were recorded every 3 days. Surviving dams were sacrificed on day 21 of gestation and fetuses examined for external, visceral and skeletal abnormalities.

There were no treatment-related findings. The small group sizes preclude the establishment of a reliable NOAEL, but when considered with the preceding range-finding study, the results indicate that MCPA was not teratogenic or embryotoxic.

In a range-finding study conducted by Hellwig et al. (1992a), MCPA (purity 94.22%) in 0.5% w/v carboxymethylcellulose was administered by gavage to groups of 10 pregnant Wistar rats at a dose of 0, 80, 120 or 160 mg/kg bw per day from day 6 to day 15 of gestation. Clinical signs, body weight and feed consumption were recorded at various times. Blood was sampled on day 16 of gestation for the analysis of haematology and clinical chemistry parameters. Surviving dams were sacrificed at this same time and examined macroscopically. Liver and kidney weights were recorded. Fetuses (plus the placenta) were weighed and examined for external abnormalities.

There were no deaths. At the highest dose, two of five dams had vaginal haemorrhage on day 15 of gestation. Selected maternal findings are summarized in Table 26. There was a dose-related reduction in body weight gain and feed consumption, which was statistically significant ($P < 0.01$) at 120 and 160 mg/kg bw per day. Perturbations in haematology and clinical chemistry parameters, including increased enzyme activity, occurred at and above 80 mg/kg bw per day and were suggestive of anaemia and impaired liver function. The relative kidney weight of high-dose dams was approximately 9% higher ($P < 0.05$) than the control value, whereas gravid uterus weight was approximately 24% lower ($P < 0.05$). The weight of the placenta was significantly lower than the control value at 120 and 160 mg/kg bw per day. The body weight of viable fetuses was significantly lower ($P < 0.01$ or 0.05) than the control values at every dose (0.53, 0.49, 0.43 and 0.37 g at 0, 80, 120 and 160 mg/kg bw per day, respectively). There were no treatment-related fetal abnormalities.

In the main study, MCPA (purity 94.22%) in 0.5% w/v carboxymethylcellulose was administered by gavage to groups of 22–24 pregnant Wistar rats (Chbb:THOM (SPF) strain) at a dose of 0, 15, 60 or 120 mg/kg bw per day from day 6 to day 15 of gestation. Dams were observed daily throughout gestation for clinical signs of toxicity, with body weight and feed consumption recorded throughout this period. On day 20 of gestation, surviving dams were sacrificed and fetuses examined for external, visceral and skeletal abnormalities.

Table 26. Findings in maternal rats dosed with MCPA from day 6 to day 15 of gestation

Parameter	Mean value			
	0 mg/kg bw per day	80 mg/kg bw per day	120 mg/kg bw per day	160 mg/kg bw per day
Body weight gain, days 6–15 (g)	44.4	38.2	28.2**	26.1**
Feed consumption, days 6–15 (g/rat per day)	25.6	24.2	21.1**	20.1**
Haemoglobin (mmol/l)	7.29	6.87*	6.83*	6.60**
Haematocrit (l/l)	0.39	0.37*	0.036**	0.035**
Mean corpuscular volume (fl)	60.47	58.60	58.51*	58.01**
Platelets ($\times 10^9$/l)	1266	1118	1106*	920**
ALT (µkat/l)	1.24	1.48	1.74**	1.90**
AST (µkat/l)	1.50	1.83	2.29*	2.42**
ALP (µkat/l)	5.35	5.86	6.81	7.21*
Calcium (mmol/l)	3.02	2.94	2.90*	2.88**
Total protein (g/l)	69.59	63.58**	62.59**	61.57**
Globulin (g/l)	30.09	26.40**	24.66**	23.69**
Triglycerides (mmol/l)	4.99	3.92	3.32**	2.60**
Cholesterol (mmol/l)	2.39	1.95**	1.62**	1.58**
Relative kidney weight (%)	0.66	0.66	0.68	0.72*
Gravid uterus weight (g)	21.6	21.6	18.2	16.4*
Placenta weight (from viable fetuses) (g)	0.26	0.25	0.24*	0.20**

From Hellwig et al. (1992a)
* $P < 0.05$; ** $P < 0.01$

There were no deaths and no treatment-related clinical signs. At 120 mg/kg bw per day, body weight gain was approximately 23% lower ($P < 0.01$) than the control value over the dosing period, concomitant with significantly lower ($P < 0.01$ or 0.05) feed consumption (up to 17% lower than the control value). The lower body weight gain and feed consumption were evident within 2 days of the commencement of dosing (days 6–8 body weight gain = 4.1 g versus 7.8 g in the controls, $P < 0.01$; days 6–8 feed consumption = 21.1 g versus 24.8 g in the controls, $P < 0.01$). Body weight gain and feed consumption were unremarkable at 15 and 60 mg/kg bw per day. There were no treatment-related macroscopic findings in dams.

At 120 mg/kg bw per day, mean placenta weight was approximately 10% lower than the control value, but was statistically significant ($P < 0.05$) only for placentas belonging to female fetuses. Also at 120 mg/kg bw per day, mean fetal body weight was approximately 12% lower ($P < 0.01$) than the control value.

External examination revealed severe malformations of the head in two high-dose fetuses (brachygnathia, microglossia, unilateral anophthalmia, proboscis, aglosstomia, caudal displacement of the left ear, bilateral anophthalmia and hydrocephaly). The study author noted that proboscis is a very rare malformation and was likely to have arisen spontaneously. There was no overall treatment-related increase in the incidence of soft tissues or skeletal malformations. Also at the highest dose was a significant ($P < 0.05$) increase in the number of fetuses with an incompletely ossified skull or incompletely or unossified sternebrae (Table 27); this may be attributable to the reduced fetal body weight at this dose.

Table 27. Incidence of skeletal retardations in fetuses

Finding	Incidence (%)			
	0 mg/kg bw per day	15 mg/kg bw per day	60 mg/kg bw per day	120 mg/kg bw per day
Skull incompletely ossified				
Fetal incidence	0.6	0	1.9	7.4**
Litter incidence	4.3	0	9.1	30*
Sternebrae not ossified				
Fetal incidence	6.2	3.4	9.4	45**
Litter incidence	30	21	32	78**
Sternebrae incompletely ossified or reduced in size				
Fetal incidence	22	9.1**	21	39**
Litter incidence	65	50	64	83
Sternebrae: only one ossification centre				
Fetal incidence	5	11	11	16**
Litter incidence	26	50	64*	61*
Total skeletal retardation				
Fetal incidence	63	57*	59	84**
Litter incidence	91	100	95	91

From Hellwig & Hildebrand (1993a)
* $P < 0.05$; ** $P < 0.01$

The NOAEL for maternal toxicity was 60 mg/kg bw per day, based on reduced body weight gain and feed consumption at 120 mg/kg bw per day. The NOAEL for fetal toxicity was also 60 mg/kg bw per day, based on reduced fetal weight and delayed skeletal maturation at 120 mg/kg bw per day (Hellwig & Hildebrand, 1993a).

MCPA 2-EHE (purity 99.9%) in 0.5% w/v aqueous carboxymethylcellulose was administered by gavage to groups of 25 pregnant SD rats (Crl:CD®(SD)BR strain) from days 6 to 19 of gestation at a dose of 0, 15, 40 or 120 mg/kg bw per day expressed as acid equivalents. Dams were observed daily throughout gestation for clinical signs. Body weight and feed consumption were recorded during gestation. Surviving dams were sacrificed on day 20 of gestation and fetuses weighed and examined for external malformations. The numbers of corpora lutea and implantation sites were determined. Maternal kidney and brain weights were recorded. Half of the fetuses were prepared and examined for visceral abnormalities, with the remainder examined for skeletal abnormalities.

There were no deaths or treatment-related clinical signs. At the highest dose, maternal body weight gain and feed consumption were significantly lower ($P < 0.01$ or 0.05) than the control values across the entire treatment period (approximately 39% and 13% lower, respectively). The lower body weight gain and feed consumption were evident within 1 day of the commencement of dosing (days 6–7 body weight gain = −3 g versus 2 g in the controls, $P < 0.01$; days 6–7 feed consumption = 19 g versus 22 g in the controls, $P < 0.01$). Also at the highest dose, relative kidney and brain weights were significantly higher ($P < 0.01$) than the control values (kidney: 0.62 g/100 g versus 0.53 g/100 g in the controls; brain: 0.53 g/100 g versus 0.46 g/100 g in the controls), which was attributable to the lower terminal body weight of this group.

Two whole-litter resorptions occurred at 120 mg/kg bw per day. Significantly increased ($P <$ 0.01) post-implantation losses (mainly early resorptions) occurred at 120 mg/kg bw per day (15.1% per litter versus 3.5% in the controls; historical control maximum = 13.5%, not statistically

significant), concomitant with a decrease in viable litter size (approximately 85% versus 97% in the controls; not statistically significant). Mean fetal body weight at 120 mg/kg bw per day (2.5 g) was significantly lower ($P < 0.01$) than the control value (3.3 g).

The incidence of malformations (external, visceral and skeletal) was increased at 120 mg/kg bw per day (5.7% versus 0.2% per litter in the control). Treatment-related fetal observations included hydrocephaly (one fetus from each of two litters at 120 mg/kg bw per day) and bent scapula and/or humerus bones (13 fetuses from two litters at 120 mg/kg bw per day). Both of these findings exceeded the performing laboratory's historical control range (0.6% versus 0.3% per litter; 4.9% versus 0.5% per litter, respectively).

The incidence of skeletal variations was significantly increased ($P < 0.01$) at 120 mg/kg bw per day (71.8% versus 35.3% in the control). This was due predominantly to a significant increase ($P < 0.01$) in the incidence of delayed ossification (sternebra 5 or 6 unossified: 61.5% versus 17% per litter in the controls; sternebra 1, 2, 3 or 4 unossified: 9.8% versus 0% per litter in the controls) and bent ribs (6.9% versus 0.3% per litter in the controls; $P < 0.05$).

The NOAEL for maternal toxicity was 40 mg/kg bw per day expressed as acid equivalents, based on reduced body weight gain and feed consumption at 120 mg/kg bw per day expressed as acid equivalents. The NOAEL for fetal toxicity was also 40 mg/kg bw per day expressed as acid equivalents, based on increased post-implantation loss, reduced fetal body weight and fetal anomalies (mainly delayed ossification) at 120 mg/kg bw per day expressed as acid equivalents (Cappon, 1999a).

MCPA DMA (purity 78.2%) in 0.5% w/v aqueous carboxymethylcellulose was administered by gavage to groups of 25 pregnant SD rats (Crl:CD®(SD)BR strain) from days 6 to 19 of gestation at a dose of 0, 15, 50 or 150 mg/kg bw per day expressed as acid equivalents. The study design was identical to that described in the preceding study on MCPA 2-EHE.

At 150 mg/kg bw per day, one dam was euthanized in extremis on day 12 of gestation. All remaining dams survived until scheduled termination. Clinical signs observed only at the highest dose during the dosing period included yellow or brown matting or staining of the fur in the urogenital region (up to seven dams), decreased defecation (four dams) and abnormal gait (five dams). Post-dosing clinical signs included wiping the mouth or face on the sides of the cage (all high-dose dams).

At the highest dose, maternal body weight gain and feed consumption were significantly lower ($P < 0.01$ or 0.05) than the control values across the entire treatment period (approximately 57% and 22% lower, respectively). The lower body weight gain and feed consumption were evident within 1 day of the commencement of dosing (days 6–7 body weight gain = −4 g versus 1 g in the controls, $P < 0.01$; days 6–7 feed consumption = 17 g versus 21 g in the controls, $P < 0.01$). Also at the highest dose, mean absolute and relative kidney weights were approximately 10% higher than the control group values; these differences were statistically significant ($P < 0.01$).

Five whole-litter resorptions (mainly early resorptions) occurred at 150 mg/kg bw per day. Significantly increased ($P < 0.01$) post-implantation losses occurred at 150 mg/kg bw per day (41.8% versus 3.8% per litter in the controls; performing laboratory's historical control maximum = 13.5% per litter), concomitant with a significant decrease ($P < 0.01$) in viable litter size (58.3% versus 96.2% in the controls; performing laboratory's historical control minimum = 86.5% per litter). Mean fetal body weight at 150 mg/kg bw per day (2.1 g) was significantly lower ($P < 0.01$) than the control value (3.5 g), which was lower than the performing laboratory's historical control minimum of 3.3 g.

Total malformations were significantly elevated ($P < 0.01$) at the highest dose (0.8%, 0%, 0% and 23.4% per litter at 0, 15, 50 and 150 mg/kg bw per day, respectively). Three fetuses from three litters in the control group and six fetuses from five litters at 150 mg/kg bw per day had external malformations. The authors noted the occurrence of localized oedema of the neck and/or thorax in three high-dose fetuses (4.4%), which was above the historical control maximum.

The incidence of skeletal malformations was increased at 150 mg/kg bw per day (23% of fetuses per litter versus 0% in the controls). Bent limb bones (scapula, humerus and/or clavicle) and rib abnormalities (fused ribs) were significantly increased ($P < 0.01$ or 0.05) at 150 mg/kg bw per day: 22.6% and 3.3% per litter, respectively, versus 0% in the controls, which exceeded the performing laboratory's historical control maxima of 0.5% and 0.6% per litter, respectively.

The incidence of skeletal variations was also significantly increased ($P < 0.01$ or 0.05) at 150 mg/kg bw per day (90.4% versus 41.8% in the controls). These variations included the following: sternebra 5 and/or 6 unossified (79.8% versus 21.4% in the controls, $P < 0.01$); 14th rudimentary rib (14.9% versus 2.5% in the controls, $P < 0.01$); sternebra 1, 2, 3 and/or 4 unossified (40% versus 0.5% in the controls, $P < 0.01$); bent rib (39.3% versus 0% in the controls, $P < 0.01$); and entire sternum unossified (5.9% versus 0.2% in the controls, $P < 0.01$). The majority of these findings were greater than the performing laboratory's historical control range.

The NOAEL for maternal toxicity was 50 mg/kg bw per day expressed as acid equivalents, based on clinical signs, reduced body weight gain and reduced feed consumption at 150 mg/kg bw per day expressed as acid equivalents. The NOAEL for fetal toxicity was also 50 mg/kg bw per day expressed as acid equivalents, based on increased post-implantation losses, reduced fetal body weight and increased fetal anomalies at 150 mg/kg bw per day expressed as acid equivalents (Cappon, 1999b).

Rabbits

In a non-guideline, range-finding study that predated principles of GLP, MCPA (unspecified purity) in 1% w/v carboxymethylcellulose was administered by gavage to groups of five inseminated Dutch Belted rabbits at a dose of 0 (n = 10), 25 or 100 mg/kg bw per day from day 1 to day 27 post-insemination. Dams were observed daily throughout gestation for clinical signs of toxicity, and body weights were recorded every 3 days. On day 28, survivors were sacrificed and examined macroscopically. Fetuses were delivered and examined.

At 100 mg/kg bw per day, one rabbit died on day 10, preceded by ataxia and body weight loss (approximately 11%). Ataxia was observed in another high-dose rabbit that survived to scheduled termination. There were no treatment-related findings at 25 mg/kg bw per day. Pregnancy was low across all groups (6/10, 2/5 and 3/5 at 0, 25 and 100 mg/kg bw per day, respectively). At 100 mg/kg bw per day, all fetuses died in utero; however, prenatal mortality was also particularly high at 0 and 25 mg/kg bw per day. There were no treatment-related malformations or variations observed at 25 mg/kg bw per day. Fetal weight (30 g) and length (88 mm) were lower than the control values at 25 mg/kg bw per day (37 g and 94 mm, respectively) (Irvine & Tucker, 1978b).

In the main study by Irvine (1980b), conducted under similar conditions, MCPA (unspecified purity) in 1% w/v carboxymethylcellulose was administered by gavage to groups of 15–18 inseminated Dutch Belted rabbits at a dose of 0 (n = 30), 5, 12, 30 or 75 mg/kg bw per day from day 6 to day 18 post-insemination.

Deaths were observed at and above 12 mg/kg bw per day (two, three and five rabbits at 12, 30 and 75 mg/kg bw per day, respectively). The authors noted that these deaths occurred in association with a respiratory infection, which appeared to have been exacerbated by treatment. At 75 mg/kg bw per day, body weight loss occurred during the first half of the dosing period, but was comparable to the control value by the end of pregnancy. Overall (days 0–28) body weight gain was somewhat lower than the control value at the highest dose (10.6%, 10.1%, 4.8%, 9.1% and 8.6% at 0, 5, 12, 30 and 75 mg/kg bw per day, respectively). Feed consumption was unaffected by treatment.

Post-implantation loss was higher than the control value at all doses (1.1%, 2.4%, 8.8%, 3% and 16% at 0, 5, 12, 30 and 75 mg/kg bw per day, respectively). However, only at 75 mg/kg bw per day was post-implantation loss higher than the historical control range (due partly to a dam resorbing a whole litter). The mean number of fetuses per dam was slightly lower than the control value at 75 mg/kg bw per day (7.21, 6.92, 6.89, 6.86 and 6.18 at 0, 5, 12, 30 or 75 mg/kg bw per day,

respectively); however, total litter weight was unremarkable. At 75 mg/kg bw per day, fetal weight and crown–rump length were higher than the control values, but not significantly so (35.9 g versus 34.2 g and 97.5 mm versus 94.6 mm, respectively).

There were no treatment-related external, visceral or skeletal malformations.

The NOAEL for maternal toxicity was 5 mg/kg bw per day, based on mortality at and above 12 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 30 mg/kg bw per day, based on increased post-implantation losses at 75 mg/kg bw per day. MCPA was not teratogenic under the conditions of this study. Because of a number of limitations, including the possible confounding effect of the underlying respiratory illness, these NOAELs are not considered suitable for risk assessment purposes.

In a range-finding study, MCPA (purity 94.22%) in 0.5% w/v aqueous carboxymethylcellulose was administered by gavage to groups of five inseminated Himalayan rabbits (Chbb:HM outbred strain) at a dose of 0, 50, 75 or 100 mg/kg bw per day from day 7 to day 19 post-insemination. Dams were observed daily throughout gestation for clinical signs of toxicity. Body weight and feed consumption were recorded every 2 or 3 days. Blood was sampled on day 20 for the analysis of haematology and clinical chemistry parameters. Surviving dams were sacrificed on day 26 and necropsied, and the kidneys and liver were weighed. Fetuses and the placenta were weighed and examined macroscopically.

Deaths occurred at 75 and 100 mg/kg bw per day (one and two dams, respectively) between days 16 and 21 of gestation, which were preceded by a general decline in health. Feed consumption was significantly lower ($P < 0.01$) than the control value at both 75 and 100 mg/kg bw per day during the dosing and post-dosing periods (approximately 14% and 36% lower, respectively). However, only the body weight gain at 100 mg/kg bw per day was consistently lower than the control value during the treatment period (but not significantly so). There was no treatment-related haematology or clinical chemistry findings. Organ weights and necropsy findings were unremarkable. While post-implantation losses were higher than the control value (5%) at 75 and 100 mg/kg bw per day (17.9% and 17.5%, respectively), they were within the performing laboratory's historical control range of 3–23.1%. There were no treatment-related fetal abnormalities (Hellwig et al., 1992b).

In the main study by Hellwig & Hildebrand (1993b), MCPA (purity 94.22%) in 0.5% w/v aqueous carboxymethylcellulose was administered by gavage to groups of 15 pregnant Himalayan rabbits (Chbb:HM outbred strain) at a dose 0, 15, 30 or 60 mg/kg bw per day from day 7 to day 19 post-insemination. Dams were observed daily for clinical signs of toxicity. Body weights were recorded every 2 or 3 days. Survivors were sacrificed on day 29 of gestation and necropsied. Fetuses were delivered and examined for external, visceral and skeletal abnormalities.

At 60 mg/kg bw per day, one dam died on day 20, and another was sacrificed in a moribund condition on day 21. Prior to death/sacrifice, both dams exhibited clinical signs (piloerection, no defecation). At necropsy, ulcerations of the stomach mucosa were detected in both decedents. At 30 mg/kg bw per day, one dam aborted its litter on day 21 and was subsequently sacrificed. At necropsy, focal haemorrhagic oedema of the colon was observed. Blood was detected in the bedding of one dam each at 30 and 60 mg/kg bw per day. At 60 mg/kg bw per day, dams lost a mean of approximately 15 g body weight over the dosing period, while control dams gained approximately 30 g; this difference was not statistically significant. Coincident with this loss of body weight was reduced feed consumption (77.1 g), which was significantly lower ($P < 0.05$) than the control value (85.5 g). There were no other treatment-related effects detected in dams, and no indication of embryo and fetal toxicity or teratogenicity at any dose.

The NOAEL for maternal toxicity was 15 mg/kg bw per day, based on clinical signs and mortality at and above 30 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 60 mg/kg bw per day, the highest dose tested.

In a published study by Ujházy et al. (2006), MCPA (purity unspecified) in 1% w/v aqueous carboxymethylcellulose was administered to groups of 20 New Zealand White rabbits by gavage at a dose of 0, 5, 10 or 25 mg/kg bw per day from days 7 to 27 of gestation. Clinical condition was observed daily, and body weight was measured at appropriate intervals throughout the study. Dams were fed a fixed weight of diet each day (100 g). Rabbits were sacrificed on day 28 of gestation and examined macroscopically. Gravid uterine weight was recorded, and pups were weighed, sexed and examined for external abnormalities. Approximately one third of pups were used for detailed evaluation of viscera, and the remainder were subjected to skeletal examination.

The number of pregnant females at the highest dose (12/20) was lower than the desired minimum (16), which was not attributable to death or visible abortion. There was some evidence of misdosing (lung dosing) across all groups. There were no treatment-related mortalities or clinical signs. When corrected for predosing differences (body weights were unevenly distributed upon allocation and were significantly different prior to the commencement of dosing), there was no treatment-related effect on body weight. There were no treatment-related macroscopic abnormalities in dams.

At 25 mg/kg bw per day, mean fetal weight (31.26 g) was significantly lower ($P < 0.05$) than the control value (32.45 g). Placental weight was significantly lower ($P < 0.05$) than the control value at 5 and 25 mg/kg bw per day (5.38, 4.92, 5.40 and 5.07 g at 0, 5, 10 and 25 mg/kg bw per day, respectively). The authors attributed these differences in body and placental weights to the relatively low body weight of dams allocated to the high-dose group, noting that they were within the range of biological variability. On this basis, these statistically significant differences are not considered to be treatment related.

No skeletal or visceral malformations were detected. In some bones (skull and pelvis), the incidence of delayed ossification was significantly higher ($P < 0.01$) than in the controls, whereas in other bones (sternebrae, forelimbs and hindlimbs), there was no difference relative to the controls, which had a relatively high background incidence of delayed ossification. As the occurrence of delayed ossification across all groups was highly variable and demonstrated no consistent pattern, it was not considered to be treatment related.

The NOAEL for maternal and embryo and fetal toxicity was 25 mg/kg bw per day, the highest dose tested. However, because of a number of limitations to the study, this NOAEL is not considered to be suitable for risk assessment purposes.

(c) In vitro studies

In a published study, inhibition of testicular deoxyribonucleic acid (DNA) synthesis by MCPA (unspecified purity) was investigated following a single gavage dose of 200 mg/kg bw to mice (unspecified strain and group size). One hour before dosing, male mice were injected intraperitoneally with 37 kBq [^{14}C]thymidine and then reinjected 3 hours after dosing with 370 kBq [^{3}H]thymidine. After a further 30 minutes, the animals were sacrificed, the testes were removed and the radioactive uptake was measured. The uptake of thymidine in the testes was inhibited by 54% ($P < 0.01$) (Seiler, 1979).

2.6 Special studies

(a) Neurotoxicity

In a published study, MCPA (purity 99%) in propylene glycol (unspecified concentration) was administered by gavage to groups of four male Sprague-Dawley (SD) rats and NMRI albino mice. Blood–brain barrier damage was examined by measuring the extravasation of albumin using the Evans blue technique. No blood–brain barrier damage was evident in mice following a single dose of 800 mg/kg bw. Following a single dose of 950 mg/kg bw, Evans blue extravasation was observed in the cerebral cortex (2/4 rats) and medulla oblongata (3/4 rats), suggesting that an acute toxic dose of MCPA can damage the blood–brain barrier in rats (Elo et al., 1988).

MCPA (purity 94.2%) in 0.5% w/v aqueous carboxymethylcellulose was administered by gavage as a single dose to groups of 10 Wistar rats (Chbb:THOM (SPF) strain) of each sex at 0, 200, 400 or 800 mg/kg bw for males and at 0, 150, 300 or 600 mg/kg bw for females and observed for 2 weeks. Rats were observed daily for clinical signs, with a more detailed examination performed weekly. Body weight was recorded weekly. A functional observational battery and motor activity assessment were performed on all rats 7 days prior to dosing and at 24 hours, 2 days and 14 days after dosing. Survivors were sacrificed after 14 days, and tissues from five rats of each sex per group were prepared for neuropathological examinations.

There were no deaths. Mean absolute body weight of high-dose males was approximately 9% and 5% lower than the control values on days 7 ($P < 0.01$) and 14 ($P < 0.05$), respectively. Body weight gain to day 7 and day 14 was approximately 48% and 12% lower ($P < 0.01$) than the control values, respectively, in high-dose males. The mean body weight gain of mid-dose males was approximately 12% lower ($P < 0.05$) than the control value to day 7. Although a similar pattern of lower body weight and body weight gain occurred in high-dose females, albeit of a smaller magnitude (approximately 3% and 20% lower than the control value, respectively), there was no significant difference relative to the control.

Clinical signs were observed only during the functional observational battery performed 24 hours after dosing and are summarized in Table 28. Ataxia and increased abdominal tension were observed at the middle and high doses, whereas impaired activity, reduced rearing and decreased righting response occurred at the highest dose. At the highest dose, motor activity was impaired in both sexes 24 hours after dosing, but not on day 7 or 14. Histopathological examinations detected no treatment-related effect on the nervous system. On this basis, the clinical signs observed during the functional observational battery are attributed to acute systemic toxicity, rather than to a direct neurotoxic effect.

Table 28. Results of functional observational battery and motor activity assessments in rats 24 hours after an acute oral dose of MCPA

Parameter	Absolute number of rats showing the effect ($n = 10$), unless otherwise specified							
	Males				Females			
	0 mg/kg bw	200 mg/kg bw	400 mg/kg bw	800 mg/kg bw	0 mg/kg bw	150 mg/kg bw	300 mg/kg bw	600 mg/kg bw
Open-field observations								
Hypoactivity	0	0	1	4	0	0	0	0
Decreased arousal	0	0	0	1	0	0	0	0
Ataxia								
- slight	0	0	2	3	0	0	0	8
- marked	0	0	1	7	0	0	0	2
Rearing (mean)	3.7	3.6	2.5	0.8**	7.8	6.2	6.3	3.8
Sensorimotor tests/reflexes								
Abdominal tension increased	0	0	2	8	0	0	4	10
Retarded righting response	0	0	0	7	0	0	0	5
Motor activity								
Overall motor activity[a]	173	161	113	65***	220	198	226	121***

From Mellert, Kaufmann & Hildebrand (1994a)

** $P < 0.01$; *** $P < 0.002$

[a] Interruptions per rat per interval.

The NOAEL was 150 mg/kg bw, based on the occurrence of clinical signs observed during the functional observational battery and impaired motor activity at 300 mg/kg bw (Mellert, Kaufmann & Hildebrand, 1994a).

MCPA 2-EHE (purity 93.5%) in 0.5% w/v aqueous carboxymethylcellulose containing Cremophore EL® was administered by gavage to groups of 10 Wistar rats (Chbb:THOM (SPF) strain) of each sex as a single dose of 0, 250, 500 or 1000 mg/kg bw (approximately equal to 0, 160, 320 and 640 mg/kg bw expressed as acid equivalents) and observed for 2 weeks. The study design was consistent with that of the preceding study by Mellert, Kaufmann & Hildebrand (1994a).

There were no deaths and no treatment-related clinical signs observed during general daily observations. In high-dose males, mean absolute body weight was approximately 8% ($P < 0.01$) and 6% ($P < 0.05$) lower than the control values on days 7 and 14, respectively, whereas body weight gain was approximately 41% ($P < 0.01$) and 20% ($P < 0.01$) lower, respectively. In high-dose females, mean absolute body weight and body weight gain were, respectively, approximately 6% ($P < 0.05$) and 43% ($P < 0.01$) lower than the control values at day 7. In mid-dose males, body weight gain was approximately 18% lower ($P < 0.01$) than the control value on day 7. The results of the functional observational battery and motor activity assessment are summarized in Table 29. At every dose, ataxia, increased abdominal tension, impaired motor activity and retarded righting reflex occurred 24 hours after administration. Hypoactivity or ataxia occurred in one of two high-dose males on day 7. There were no treatment-related neuropathological findings.

Table 29. Results of functional observational battery and motor activity assessments in rats 24 hours after an acute oral dose of MCPA 2-EHE

Parameter	Absolute number of rats showing the effect ($n = 10$), unless otherwise specified							
	Males				Females			
	0 mg/kg bw	250 mg/kg bw	500 mg/kg bw	1000 mg/kg bw	0 mg/kg bw	250 mg/kg bw	500 mg/kg bw	1000 mg/kg bw
Home cage observations								
Impaired activity (apathy)	0	0	0	2	0	0	0	0
Palpebral closure (eyelids permanently closed)	0	0	0	1	0	0	0	0
Open-field observations								
Abnormal posture (abdominal position)	0	0	0	2	0	0	0	0
Impaired activity (hypoactivity or apathy)	0	1	3	4	0	0	0	2
Impaired gait (slight or severe ataxia)	0	5	10	10	0	3	8	10
Urine staining of fur	0	0	0	0	0	0	0	1
Sensorimotor tests/reflexes								
Increased abdominal tension	0	0	3	2	0	3	7	5
Retarded righting response	0	0	4	6	0	1	0	7
Motor activity[a]	57	59	46	36	124	101	87**	67***

From Mellert, Kaufmann & Hildebrand (1994b)
** $P < 0.01$; *** $P < 0.002$
[a] Interruptions per rat per interval.

No NOAEL could be identified. The LOAEL was 250 mg/kg bw (approximately 160 mg/kg bw expressed as acid equivalents), the lowest dose tested, based on clinical signs observed during the functional observational battery and transient impaired motor activity (Mellert, Kaufmann & Hildebrand, 1994b).

In a similar study of acute neurotoxicity, MCPA DMA (purity 91.8%) in 0.5% w/v aqueous carboxymethylcellulose was administered by gavage to groups of 10 Wistar rats (Chbb:THOM (SPF) strain) of each sex as a single dose of 0, 175, 350 or 700 mg/kg bw (approximately equal to 0, 143, 287 and 574 mg/kg bw expressed as acid equivalents) and observed for 2 weeks. The study design was consistent with that of the preceding two studies (Mellert, Kaufmann & Hildebrand, 1994a,b).

There were no mortalities or clinical signs observed during general daily observations. In high-dose males, absolute body weight and body weight gain were significantly lower (approximately 4% and 26% lower, respectively; $P < 0.05$ and 0.01, respectively) than the control values on day 7. In high-dose females, body weight gain was approximately 45% lower ($P < 0.01$) than the control value on day 7. The results of the functional observational battery and motor activity assessment are summarized in Table 30. Ataxia occurred across all treated groups of females and at the middle and high doses in males. Hypoactivity and increased abdominal tension occurred at the middle and/or highest doses. Motor activity was impaired at the highest dose, but only the effect in females was statistically significant ($P < 0.002$). No abnormal functional observational battery findings or effects on motor activity occurred on day 7 or 14. There were no treatment-related neuropathological findings.

Table 30. Results of functional observational battery and motor activity assessments in rats 24 hours after an acute oral dose of MCPA DMA

Parameter	Absolute number of rats showing the effect ($n = 10$), unless otherwise specified							
	Males				Females			
	0 mg/kg bw	175 mg/kg bw	350 mg/kg bw	700 mg/kg bw	0 mg/kg bw	175 mg/kg bw	350 mg/kg bw	700 mg/kg bw
Open-field observations								
Impaired gait (ataxia)								
- severe	0	0	0	3	0	0	1	5
- slight	0	0	6	7	0	3	7	4
Impaired activity (hypoactivity)	0	0	0	5	0	0	0	1
Sensorimotor tests/reflexes								
Increased abdominal tension	0	0	0	1	0	0	1	2
Motor activity[a]	45	58	40	27	137	126	96	25***

From Mellert, Kaufmann & Hildebrand (1994c)
*** $P < 0.002$
[a] Interruptions per rat per interval.

No NOAEL could be identified. The LOAEL was 175 mg/kg bw (approximately equal to 143 mg/kg bw expressed as acid equivalents), the lowest dose tested, based on the occurrence of ataxia (Mellert, Kaufmann & Hildebrand, 1994c).

MCPA (purity 94.2%) was administered to groups of 15 Wistar rats (Chbb: THOM (SPF) strain) of each sex for 3 months at a dietary concentration of 0, 50, 500 or 2500 ppm (equal to 0, 4, 38

and 183 mg/kg bw per day, respectively). Achieved doses were 0, 3, 34 and 177 mg/kg bw per day for males and 0, 4, 42 and 188 mg/kg bw per day for females at 0, 50, 500 and 2500 ppm, respectively. Although nominally a study of neurotoxicity, the study design incorporated all the elements of a guideline subchronic toxicity study. Rats were observed daily for clinical signs, with body weight and feed consumption recorded weekly. A functional observational battery and motor activity assessment were performed on 10 rats of each sex per dose prior to the commencement of dosing and during weeks 4, 8 and 13. Blood and urine were collected from 10 rats of each sex per group at the end of the study for the analysis of standard haematology, clinical chemistry or urine analysis parameters. Ophthalmoscopy was performed on 10 rats of each sex per group at the end of the study. Following scheduled sacrifice, tissues were prepared from five rats of each sex per group for neuropathological examination. Remaining rats were macroscopically examined; organs were weighed and examined histopathologically.

One high-dose female died on day 57 due to cachexia. The only treatment-related clinical sign observed during general daily observations was paleness in 1 male and 13 females at the highest dose. At the highest dose, absolute body weight was approximately 27% and 21% lower ($P < 0.01$) than the control values in high-dose males and females, respectively, whereas overall body weight gain was approximately 42% and 48% lower ($P < 0.01$) than the control values, respectively. In mid-dose females, body weight gain to day 90 was approximately 12% lower ($P < 0.01$ or 0.05) than the control value. Feed consumption was decreased at the highest dose (approximately 15–33% lower than the control value in males and approximately 18–38% lower in females). Water consumption was increased in mid-dose females (approximately 13–41% higher than the control value) and at the high dose (approximately 22–42% higher than the control value in males and approximately 87–145% higher than the control value in females).

Treatment-related functional observational battery findings were confined to the highest dose and included paleness (two males and five females on day 85), decreased forelimb grip strength in high-dose males on day 50 (4.2 N versus 4.8 N in the control, $P < 0.01$), decreased hindlimb grip strength in females on day 85 (1.6 N versus 2.2 N, not significant) and decreased landing foot spread in males on day 22 (11 cm versus 12.5 cm in the controls, $P < 0.01$). Motor activity was approximately 20–30% lower than the control values in high-dose males and females on days 22, 50 and 85, but not significantly so. Ophthalmoscopy was unremarkable.

Selected haematology, clinical chemistry and urine analysis parameters are summarized in Table 31. Changes in red cell parameters consistent with macrocytic anaemia occurred at the highest dose (reduced erythrocytes, haemoglobin, haematocrit and mean corpuscular haemoglobin concentration). Red blood cell morphology was unremarkable. Significantly reduced ($P < 0.01$) platelet counts also occurred at the highest dose in both sexes. Significantly decreased white blood cell counts occurred in high-dose females; in the males, there was a trend towards reduced leukocyte numbers. The mechanism of the effect on red blood cells and platelets appears to be due to reduced cell production, as indicated by the histological findings in bone marrow. In high-dose females, significantly prolonged ($P < 0.01$) prothrombin times were noted. Effects on clinical chemistry parameters occurred mainly at the highest dose and included significantly reduced ($P < 0.01$ or 0.05) glucose, total proteins and globulins and increased urea, creatinine, ALT, AST and ALP activities. Serum triglyceride concentrations were significantly lower ($P < 0.01$ or 0.05) than the control values at the middle (males) and high doses (both sexes). In high-dose females, urine volume was increased and urine specific gravity significantly decreased ($P < 0.01$).

Absolute organ weights of most organs measured were lower than the control values at the highest dose, most likely due to the reduced body weight at this dose (Table 32). However, the increase in absolute liver weight in high-dose females occurred in conjunction with elevated serum enzymes and histopathology and is therefore considered treatment related. The relative kidney weights of mid- and high-dose males were significantly increased relative to the control values, with the increase in 500 ppm males not attributable to the lower body weight. This may correlate with disturbances of creatinine and urea seen in males, although no histological abnormalities in the kidneys were detected. Lower relative adrenal weights (females) and testes weights (in males) also cannot be attributed to lower body weight.

Table 31. Selected findings in rats following subchronic exposure to MCPA

Parameter	Mean value							
	Males				Females			
	0 ppm	50 ppm	500 ppm	2500 ppm	0 ppm	50 ppm	500 ppm	2500 ppm
WBC (× 10^9/l)	6.56	7.30	7.13	5.44	4.63	4.64	4.66	3.22**
RBC (× 10^{12}/l)	9.13	9.13	8.99	7.75**	7.90	7.84	7.97	6.68**
Hb (mmol/l)	9.8	9.9	9.6	8.8**	9.2	9.0	9.2	8.0**
Hct (l/l)	0.469	0.473	0.466	0.437**	0.423	0.421	0.424	0.386**
MCV (fl)	51.4	51.8	51.8	56.5**	53.6	53.7	53.2	57.9**
MCH (fmol)	1.07	1.08	1.07	1.14**	1.17	1.15	1.16	1.19
MCHC (mmol/l)	20.81	20.91	20.60	20.16*	21.84	21.46	21.74	20.60**
Platelets (× 10^9/l)	781	757	788	591**	736	747	655	601**
Prothrombin time (s)	30.5	31.3	30.0	31.5	27.1	27.7	26.9	29.2**
ALT (µkat/l)	1.11	1.07	2.32	5.33**	0.88	0.81	1.32	2.38**
AST (µkat/l)	1.82	1.81	2.79	5.31**	1.64	1.48	2.06	2.63**
ALP (µkat/l)	4.46	4.73	4.69	9.22**	3.58	3.70	3.89	5.87**
Chloride (mmol/l)	104.8	103.9	105.3	104.1	107.9	106.0	106.6	103.2**
Calcium (mmol/l)	2.89	2.94	2.89	2.75**	2.77	2.77	2.77	2.57**
Urea (mmol/l)	6.55	6.46	6.36	7.22*	7.48	7.52	8.47*	6.91
Creatinine (µmol/l)	54.7	57.9	58.6	66.7**	50.8	52.8	55.6*	54.9
Glucose (mmol/l)	7.98	7.53	7.66	6.36**	7.36	7.18	7.26	6.13*
Total protein (g/l)	66.46	68.34	67.43	61.47*	64.42	63.47	64.55	60.18**
Globulin (g/l)	34.25	35.57	34.81	29.72**	30.20	29.65	30.03	28.12
Triglycerides (mmol/l)	4.15	3.94	2.82*	0.64**	2.50	2.30	2.19	0.65**
Cholesterol (mmol/l)	1.88	1.96	1.92	1.71	2.00	1.95	2.07	2.38*
Urine volume (ml)	5.5	6.4	5.3	4.7	3.1	2.7	3.0	4.5
Urine specific gravity (g/l)								
- < 1040	3	7	4	6	2	0	1	8**
- > 1040	7	3	6	4	8	10	9	1

From Mellert et al. (1994b)

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Hb, haemoglobin; Hct, haematocrit; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cells; WBC, white blood cells; * $P < 0.05$; ** $P < 0.01$

Gross pathological findings occurred mainly at the highest dose and included the following: prominent acinar pattern of the liver (0/10, 2/10, 0/10 and 6/10 males and 0/10, 1/10, 1/10 and 8/10 females at 0, 50, 500 and 2500 ppm, respectively); dilatation of the heart (0/10, 5/10, 3/10 and 10/10 males and 0/10, 1/10, 0/10 and 7/10 females at 0, 50, 500 and 2500 ppm, respectively); reduced kidney (2 high-dose males), testes (all high-dose males), epididymides (5 high-dose males) and seminal vesicle sizes (2 high-dose males); and discoloration of the adrenal cortex (10 high-dose males and 5 high-dose females).

Table 32. Organ weights in rats following subchronic exposure to MCPA

Organ	Mean value							
	Males				Females			
	0 ppm	50 ppm	500 ppm	2500 ppm	0 ppm	50 ppm	500 ppm	2500 ppm
Absolute organ weights (g)								
Liver	16.063	14.848	14.415	11.471**	7.86	7.489	7.393	8.12
Kidneys	3.165	3.207	3.471	2.513**	1.919	1.877	1.883	1.588**
Testes	3.63	3.739	3.582	1.555**	—	—	—	—
Brain	2.04	2.058	2.053	1.963	1.837	1.802	1.809	1.819
Adrenals	0.0813	0.087	0.0854	0.0604**	0.1059	0.1117	0.103	0.063**
Heart	1.444	1.401	1.36	1.045**	0.912	0.937	0.897	0.806**
Relative organ weights (%)								
Liver	3.391	3.198	3.146	3.552	3.193	3.045	3.145	4.233**
Kidneys	0.672	0.696	0.76*	0.773*	0.78	0.765	0.801	0.824
Testes	0.774	0.811	0.787	0.478**	—	—	—	—
Brain	0.434	0.449	0.452	0.609**	0.748	0.734	0.772	0.52**
Adrenals	0.017	0.019	0.019	0.019	0.043	0.046	0.044	0.033**
Heart	0.306	0.305	0.299	0.323	0.371	0.381	0.382	0.42**

From Mellert et al. (1994b)
* $P < 0.05$; ** $P < 0.01$

Histopathology revealed marked hepatocellular cytoplasmic eosinophilia and granular cytoplasm (moderate or severe) in the liver of all high-dose males and nine high-dose females. Single cases of anisokaryosis and increased mitosis (slight) were observed in the liver of high-dose males. Bile duct hyperplasia (minimal) occurred in two high-dose males. Foam cells (minimal to moderate) were detected in the lungs of mid- and high-dose males (one and eight rats, respectively, versus zero in the controls) and high-dose females (nine versus zero in the controls). Hypocellularity of the bone marrow (slight or moderate) (five high-dose females) and hypocellularity of the marrow from the cervical (three high-dose females), thoracic (three high-dose females) and lumbar cord (three high-dose females) and sternum (six high-dose males and six high-dose females, minimal to severe) were consistent with the perturbations in haematological parameters, suggesting impaired red cell production. Increased lipid storage in the adrenal cortex (minimal to severe) was the only histological finding in this tissue, despite markedly lower adrenal weights (1/10, 0/10, 7/10 and 10/10 males and 1/10, 0/10, 6/10 and 9/10 females at 0, 50, 500 and 2500 ppm, respectively). Diffuse atrophy of the testes (graded as severe or extreme) occurred in all high-dose males. Oligozoospermia and aspermia (extreme) were detected in the epididymis at the highest dose (three and seven rats, respectively). Thymic atrophy (moderate or severe) occurred in three high-dose females. There were no treatment-related neuropathological findings.

The NOAEL was 50 ppm (equal to 3 mg/kg bw per day), based on reduced body weight gain in females, increased relative kidney weight in males and increased adrenal lipid in both sexes at 500 ppm (approximately equal to 34 mg/kg bw per day) (Mellert et al., 1994b).

MCPA 2-EHE (purity 93.5%) was admixed in the diet and fed to groups of 15 Wistar rats (Chbb: THOM (SPF) strain) of each sex at a concentration of 0, 75, 750 or 3750 ppm for 13 weeks. The study design and dose regimen (in terms of acid equivalents) were consistent with the previous study by Mellert et al. (1994b). Achieved doses were 0, 5, 54 and 261 mg/kg bw per day for males (approximately equal to 0, 3.2, 34, 56 and 167 mg/kg bw per day expressed as acid equivalents) and 0,

6, 63 and 296 mg/kg bw per day for females (approximately equal to 0, 3.8, 40 and 189 mg/kg bw per day expressed as acid equivalents) at 0, 75, 750 and 3750 ppm, respectively.

There were no mortalities. Clinical signs observed at the highest dose included paleness (one high-dose male and six high-dose females) and cataracts (two high-dose males and one high-dose female). At the highest dose, significantly lower body weight (approximately 34% and 12% lower than the control values in males and females, respectively, on day 91; $P < 0.01$) and overall body weight gain (53% and 28% lower than the control values in males and females, respectively; $P < 0.01$) occurred. At the middle dose, body weight and body weight gain were approximately 8% lower than the control values in males (significant [$P < 0.05$] only in rats used for the functional observational battery on days 53 and 88). Feed consumption was approximately 25–32% and 10–28% lower than the control values in males and females, respectively. Water consumption was significantly increased in mid-dose females (approximately 23–38% higher than the control value) and in both sexes at the highest dose (9–38% higher than the control value in males and 67–112% higher in females).

Home cage, open-field and sensorimotor observations were unremarkable. The forelimb grip strength of high-dose males (4.2 N) was significantly lower ($P < 0.01$) than the control value (5.4 N) on day 88. At the highest dose, landing foot splay (day 25) was significantly lower ($P < 0.01$ or 0.002) than the control value in both sexes (10.8 cm versus 12.6 cm for controls in males; 10.0 cm versus 11.7 cm for controls in females). In males, overall motor activity was significantly lower than the control values at the middle and high doses on day 25 (218, 205, 165** and 144** N at 0, 75, 750 and 3750 ppm, respectively; ** $P < 0.01$) and at every dose on day 88 (182, 138*, 122*** and 107*** N at 0, 75, 750 and 3750 ppm, respectively; * $P < 0.05$ and ** $P < 0.001$). In females, significantly lower ($P < 0.05$, 0.01 or 0.02) motor activity occurred at the highest dose on days 25, 53 and 88 (170 N versus 264 N, 169 N versus 329 N and 153 N versus 257 N in the controls, respectively).

Ophthalmoscopic abnormalities were detected at day 90 or 91 in high-dose rats and included cataracts (2/15 males and 1/15 females), opacity (3/15 males and 3/15 females) and striate thickening of the lens star (11/15 females).

Selected haematology, clinical chemistry and urine analysis parameters are summarized in Table 33. At the end of the study, significantly decreased ($P < 0.01$) red blood cells, mean corpuscular haemoglobin concentration and platelet counts and significantly increased ($P < 0.01$) mean corpuscular volume and mean corpuscular haemoglobin occurred at the highest dose. The anaemia was considered to be a consequence of myeloid atrophy of bone marrow, seen histologically as hypocellularity. Additionally, significantly reduced ($P < 0.01$) haemoglobin and haematocrit occurred in high-dose females. White blood cell count was significantly lower ($P < 0.01$) than the control value in high-dose males. Prothrombin time was significantly increased ($P < 0.01$) in high-dose males. Plasma ALT and ALP were significantly elevated ($P < 0.01$ or 0.05) at the highest dose, with AST also significantly elevated ($P < 0.05$) in high-dose females. Treatment-related effects on clinical chemistry parameters included significantly decreased ($P < 0.01$ or 0.05) calcium, glucose, total bilirubin and triglycerides (both sexes at the highest dose), decreased ($P < 0.01$) inorganic phosphate, total protein and globulin (high-dose males) and increased ($P < 0.01$) serum creatinine (mid- and high-dose females, high-dose males). At the highest dose, there was an increase in urine volume in females (not significant) and an increased number of rats voiding urine with lower specific gravity (not significant).

Treatment-related macroscopic abnormalities included reduced testes and epididymides size (10/10 and 8/10 high-dose males, respectively) and cataracts (2/10 high-dose males). Absolute and relative organ weight findings are presented in Table 34. The absolute organ weights of high-dose males were all significantly lower ($P < 0.01$) than the control values. The magnitude of the difference was approximately 8% for brain, approximately 25% for liver, kidney and heart and approximately 37% for testes and adrenals. With the exception of the adrenals, all corresponding relative organ weights were significantly higher ($P < 0.01$) than the control values (< 20% for liver, kidney and heart, approximately 35% for testes and approximately 45% for brain). The relative kidney weight of mid-dose males was also significantly higher than the control value. Fewer absolute organ weight differences were detected in high-dose females; absolute brain ($P < 0.05$) and adrenal weights ($P <$

0.01) were approximately 5% and 36% lower than the control values, respectively. However, all relative organ weights were significantly lower ($P < 0.01$ or 0.05) than the control values (< 20% lower for kidneys, heart and brain, approximately 36% for liver and 22% for adrenals). Corroborative pathology was detected only in the liver and testes, and therefore all other organ weight differences are attributable to the lower terminal body weight at the highest dose.

Table 33. Selected findings in rats following subchronic exposure to MCPA 2-EHE

Parameter	Mean value							
	Males				Females			
	0 ppm	75 ppm	750 ppm	3750 ppm	0 ppm	75 ppm	750 ppm	3750 ppm
WBC ($\times 10^9$/l)	6.31	5.68	5.31	3.54**	3.65	3.94	3.88	3.54
RBC ($\times 10^{12}$/l)	8.62	8.71	8.99	7.74**	8.24	8.50	8.08	7.09**
Hb (mmol/l)	9.2	9.4	9.6	8.8	9.1	9.3	9.1	8.1**
Hct (l/l)	0.454	0.455	0.464	0.446	0.443	0.455	0.434	0.408**
MCV (fl)	52.7	52.3	51.7	57.7**	53.7	53.5	53.8	57.6**
MCH (fmol)	1.07	1.08	1.06	1.14**	1.11	1.10	1.12	1.15*
MCHC (mmol/l)	20.30	20.62	20.58	19.73*	20.59	20.51	20.83	19.86**
Platelets ($\times 10^9$/l)	745	748	733	473**	756	816	788	577**
Prothrombin time (s)	30.4	30.1	31.1	33.4*	27.5	27.7	27.2	28.6
ALT (µkat/l)	1.36	1.13	1.30	2.59**	0.89	0.89	0.89	1.66*
AST (µkat/l)	2.63	1.84	1.99	3.15	1.76	1.57	1.62	2.26*
ALP (µkat/l)	5.17	4.67	4.82	9.56**	4.18	4.10	4.27	6.29**
Inorganic phosphorus (mmol/l)	2.19	2.04	2.05	1.83**	1.90	1.76	1.86	1.93
Calcium (mmol/l)	2.80	2.80	2.76	2.65**	2.70	2.74	2.74	2.61*
Creatinine (µmol/l)	55.2	51.7	51.6	62.9**	51.1	52.6	58.1**	59.1**
Glucose (mmol/l)	8.10	7.73	7.56	5.92**	7.50	7.80	7.44	5.83**
Total bilirubin (µmol/l)	3.47	2.94	2.96	2.56*	2.72	2.87	2.98	2.07*
Total protein (g/l)	63.94	63.54	62.34	57.63**	62.42	63.79	64.92	60.72
Globulin (g/l)	32.72	32.31	31.31	26.79**	28.20	29.14	29.96	27.82
Triglycerides (mmol/l)	3.14	3.37	3.71	0.57**	2.05	2.27	2.05	0.82**
Urine volume (ml)	4.1	3.9	4.3	3.7	2.0	2.3	2.6	3.6
Urine specific gravity (g/l)								
- < 1040	1	1	3	5	3	1	1	4
- > 1040	9	9	7	5	7	9	9	6

From Mellert et al. (1994c)
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Hb, haemoglobin; Hct, haematocrit; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cells; WBC, white blood cells; * $P < 0.05$; ** $P < 0.01$

Histopathology revealed cytoplasmic eosinophilia and granular cytoplasm of hepatocytes (slight to severe) in all high-dose rats (0/10 in the controls). Foam cell accumulations (mainly slight) were observed in the lung of all high-dose rats (minimal observed in one control male). Severe diffuse testicular atrophy (10/10 rats), with associated oligozoospermia (3/10 rats) or aspermia (7/10 rats) in epididymides, was reported in high-dose males (0/10 in the controls). Focal testicular atrophy (minimal to moderate) was observed in four mid-dose males (0/10 in the controls). Hypocellularity of

the bone marrow (grade minimal to moderate) was detected at a number of sites, including the cervical (one high-dose male), thoracic (three high-dose males and females) and lumbar cord (0/10, 0/10, 2/10 and 7/10 males and 1/10, 0/10, 2/10 and 6/10 females at 0, 75, 750 and 3750 ppm, respectively) and sternum (0/10, 0/10, 1/10 and 9/10 males and 2/10, 0/10, 0/10 and 8/10 females at 0, 75, 750 and 3750 ppm, respectively). Hypocellularity of the bone marrow from the femur of high-dose rats was somewhat variable; in males, hypocellularity occurred with similar frequency to the controls, whereas in females, the incidence was increased (3/10, 0/10, 0/10 and 8/10 at 0, 75, 750 and 3750 ppm, respectively). There were no treatment-related microscopic findings in the adrenals, as occurred in the parallel study on MCPA acid. There were no treatment-related neuropathological findings.

Table 34. Organ weights in rats following subchronic exposure to MCPA 2-EHE

Organ	Mean value							
	Males				Females			
	0 ppm	75 ppm	750 ppm	3750 ppm	0 ppm	75 ppm	750 ppm	3750 ppm
Absolute organ weights (g)								
Liver	15.15	15.06	14.4	11.32**	7.86	7.51	7.48	8.71
Kidneys	3.06	3.13	3.25	2.28**	1.98	1.94	1.90	1.85
Testes	3.52	3.79	3.60	1.48**	—	—	—	—
Heart	1.41	1.48	1.39	1.09**	0.95	0.97	0.97	0.92
Brain	2.06	2.11	2.09	1.91**	1.94	1.90	1.91	1.85*
Adrenals	0.086	0.083	0.088	0.055**	0.11	0.10	0.11	0.07**
Relative organ weights (%)								
Liver	3.24	3.28	3.25	3.75**	3.01	2.95	2.94	3.97**
Kidneys	0.65	0.69	0.74**	0.75**	0.76	0.76	0.75	0.84*
Testes	0.76	0.83	0.82	0.49**	—	—	—	—
Heart	0.30	0.32	0.32	0.36**	0.37	0.38	0.38	0.42**
Brain	0.44	0.46	0.47	0.64**	0.75	0.75	0.76	0.85**
Adrenals	0.018	0.18	0.02	0.018	0.041	0.39	0.043	0.032**

From Mellert et al. (1994c)
* $P < 0.05$; ** $P < 0.01$

The NOAEL was 75 ppm (approximately equal to 3 mg/kg bw per day expressed as acid equivalents), based on decreased motor activity, increased serum creatinine and focal testicular atrophy at 750 ppm (approximately equal to 34 mg/kg bw per day expressed as acid equivalents) (Mellert et al., 1994c).

MCPA DMA (purity 91.8%) was admixed in the diet at a concentration of 0, 60, 600 or 3000 ppm and fed to Wistar rats (Chbb:THOM (SPF) strain) under the same experimental conditions (and at similar doses in terms of acid equivalents) described in Mellert et al. (1994b,c). Achieved doses were 0, 4, 42 and 208 mg/kg bw per day for males (approximately equal to 0, 3.3, 34 and 170 mg/kg bw per day expressed as acid equivalents) and 0, 5, 48 and 232 mg/kg bw per day for females (approximately equal to 0, 4, 40 and 190 mg/kg bw per day expressed as acid equivalents) at 0, 60, 600 and 3000 ppm, respectively.

There were no deaths and no treatment-related clinical signs. At the highest dose, reduced feed consumption (10–18% and 6–21% lower than the control values in males and females, respectively), lower absolute body weight (significantly lower [$P < 0.01$] than the control values in both sexes at every week; approximately 15% lower than the control value on day 91) and body

weight gain (significantly lower [$P < 0.01$] than the control values during every week; 26% and 32% lower in males and females, respectively) occurred. Water consumption was not recorded. The functional observational battery was generally unremarkable, with the exception of significantly reduced ($P < 0.01$) forelimb grip strength in high-dose females on day 87 (3.5 N versus 4.5 N in the controls). There was no effect on motor activity. Ophthalmoscopy was unremarkable.

Selected haematology, clinical chemistry and urine analysis parameters are summarized in Table 35. Significantly reduced ($P < 0.01$) white blood cells, red blood cells, haemoglobin and haematocrit occurred in high-dose females, whereas significantly reduced ($P < 0.01$) platelets and mean corpuscular volume occurred in both sexes. These treatment-related effects were attributable to a hypoplastic or hypoproliferative anaemia, confirmed by histopathological findings in the bone marrow. Perturbation in clinical chemistry parameters at the highest dose included the following: significantly decreased ($P < 0.01$) serum calcium, triglycerides (both sexes) and glucose (females); significantly increased ($P < 0.01$) ALT, AST, ALP and creatinine (both sexes); and significantly increased ($P < 0.01$ or 0.05) GGT, serum cholesterol and magnesium (males). Serum creatinine was also elevated ($P < 0.01$) in mid-dose females. Urine volume was increased and urine specific gravity decreased in high-dose females (not statistically significant).

Table 35. Selected findings in rats following subchronic exposure to MCPA DMA

Parameter	Mean value							
	Males				Females			
	0 ppm	60 ppm	600 ppm	3000 ppm	0 ppm	60 ppm	600 ppm	3000 ppm
WBC ($\times 10^9$/l)	6.83	7.92	7.57	6.81	4.14	4.56	4.73	3.29*
RBC ($\times 10^{12}$/l)	8.64	8.78	8.77	8.35	8.20	8.37	8.12	7.18**
Hb (mmol/l)	9.2	9.5	9.5	9.2	9.2	9.4	9.1	8.3*
Hct (l/l)	0.440	0.453	0.454	0.443	0.439	0.448	0.432	0.400**
MCV (fl)	51.0	51.6	51.8	53.2**	53.5	53.6	53.3	55.8**
Platelets ($\times 10^9$/l)	759	729	740	573**	800	735	776	607**
ALT (µkat/l)	1.05	0.87	1.43	10.52**	0.90	0.80	1.01	3.08*
AST (µkat/l)	1.65	1.49	2.01	8.78**	1.53	1.69	2.01	3.52**
ALP (µkat/l)	4.97	5.14	5.17	8.45**	4.03	4.21	4.23	5.55**
GGT (nkat/l)	0	0	2	9**	8	11	2	8
Chloride (mmol/l)	103.2	103.3	102.3	101.0*	105.0	104.6	105.0	103.4
Calcium (mmol/l)	2.70	2.71	2.73	2.60**	2.84	2.90	2.87	2.69**
Creatinine (µmol/l)	58.8	57.9	60.0	68.9**	53.5	55.9	57.4*	58.7**
Glucose (mmol/l)	8.29	7.86	8.26	7.84	7.32	7.04	7.26	5.94**
Total bilirubin (µmol/l)	2.63	2.75	2.78	2.51	2.59	2.95	2.71	1.86*
Triglycerides (mmol/l)	5.57	4.80	4.48	1.54**	3.04	3.02	3.60	0.95**
Cholesterol (mmol/l)	1.73	1.87	1.83	2.52**	1.74	1.70	1.69	1.91
Magnesium (mmol/l)	0.83	0.80	0.84	0.91**	0.91	0.94	0.92	0.89
Urine volume (ml)	5.2	5.1	6.0	4.8	1.6	1.9	2.6	3.1
Urine specific gravity (g/l)								
- < 1040	0	3	5*	2	0	0	1	3
- > 1040	10	7	5	8	10	10	9	7

From Mellert et al. (1994a)

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transpeptidase; Hb, haemoglobin; Hct, haematocrit; MCV, mean corpuscular volume; RBC, red blood cells; WBC, white blood cells; * $P < 0.05$; ** $P < 0.01$

There were no treatment-related macroscopic abnormalities. Absolute and relative organ weights are presented in Table 36. In most instances, significant differences in absolute and/or relative organ weights reflect the body weight differences between treated and control groups. The one exception to this is the significant increase ($P < 0.01$) in relative liver weight in high-dose females compared with the controls (approximately 20% increase in size), concomitant with increased liver enzymes (see Table 35) and histopathology (discussed below).

Table 36. Organ weights in rats following subchronic exposure to MCPA 2-EHE

Organ	Mean value							
	Males				Females			
	0 ppm	60 ppm	600 ppm	3000 ppm	0 ppm	60 ppm	600 ppm	3000 ppm
Absolute organ weights (g)								
Liver	17.00	14.53*	14.18*	11.88**	7.64	7.64	7.08	7.68
Kidneys	3.19	3.21	3.35	3.20	1.97	1.98	1.95	1.75*
Testes	3.78	3.82	3.69	3.35	—	—	—	—
Heart	1.61	1.58	1.60	1.35**	0.96	1.01	0.96	0.85*
Brain	2.15	2.07*	2.11	2.04**	1.87	1.86	1.89	1.86
Adrenals	0.096	0.095	0.092	0.077*	0.12	0.10	0.095	0.068*
Relative organ weights (%)								
Liver	3.44	3.12*	3.04*	2.94**	2.95	3.00	2.89	3.61**
Kidneys	0.65	0.69	0.72*	0.79**	0.76	0.78	0.80	0.82
Testes	0.77	0.83	0.80	0.83	—	—	—	—
Heart	0.33	0.34	0.34	0.33	0.37	0.40	0.39	0.40
Brain	0.44	0.45	0.45	0.51**	0.73	0.73	0.78	0.88**
Adrenals	0.02	0.02	0.02	0.02	0.043	0.041	0.039	0.032**

From Mellert et al. (1994a)
* $P < 0.05$; ** $P < 0.01$

Treatment-related histopathological findings were confined to the highest dose. In the liver, slight eosinophilia occurred in 10 of 10 females, and minimal to slight single-cell necrosis was detected in 10 of 10 males and 9 of 10 females. Minimal bile duct hyperplasia occurred in 5 of 10 females. Foam cell aggregates in the lung (minimal to moderate) were observed in rats of both sexes (10/10 males and 9/10 females). Diffuse testicular atrophy was detected in 2 of 10 males (one of these graded as severe), with oligozoospermia in the epididymides of the rat with severe atrophy. Hypocellularity of the bone marrow occurred variably at a number of sites (bone marrow: 1/10, 0/10, 0/10 and 5/10 males and 2/10, 0/10, 0/10 and 5/10 females at 0, 60, 600 and 3000 ppm, respectively; sternum: 5/10 high-dose males and 5/10 high-dose females versus 0/10 in the controls; femur: 7/10, 0/10, 8/10 and 9/10 males and 0/10, 0/10, 0/10 and 8/10 females at 0, 60, 600 and 6000 ppm, respectively; thoracic cord: 3/10 females versus 0/10 in the controls). There were no treatment-related neuropathological findings.

The NOAEL was 60 ppm (equal to 4 mg/kg bw per day expressed as acid equivalents), based on elevated serum creatinine in females at 600 ppm (equal to 40 mg/kg bw per day expressed as acid equivalents) (Mellert et al., 1994a).

(b) Enzyme induction and peroxisome proliferation

Hietanen et al. (1983) examined the effects of phenoxyacetic acid herbicides on hepatic and intestinal drug metabolizing enzymes in male Wistar rats. MCPA (unspecified purity and vehicle) was administered by gavage to an unspecified number of rats at 100 or 150–200 mg/kg bw on 5 days/week

for 2 weeks. The 200 mg/kg bw dose was reduced to 150 mg/kg bw per day after 4 days due to deaths and clinical signs (drowsiness). Rats were killed 24 hours after the last dose, and the liver and proximal small intestine were prepared for the analysis of microsomal enzymes. Hepatic ethoxycoumarin *O*-deethylase (ECOD) and epoxide hydrolase (EH) activities were significantly increased ($P < 0.01$; approximately 2- and 1.6-fold, respectively) at both 100 and 150–200 mg/kg bw. Intestinal ECOD and EH activities were significantly increased ($P < 0.01$; < 2-fold) at 150–200 mg/kg bw; there was enzyme induction at 100 mg/kg bw. Hepatic glutathione *S*-transferase and intestinal uridine diphosphate–glucuronosyltransferase (UDPGT) activities were significantly lower ($P < 0.05$; < 2-fold) than the control values at 150–200 mg/kg bw. There was no effect on hepatic cytochrome P450, polyphenol oxidase hydroxylase or aryl hydrocarbon hydroxylase (AHH) or intestinal AHH (Hietanen et al., 1983).

In a subsequent study by Hietanen et al. (1985), an unspecified number of male Wistar rats were gavaged with MCPA (isooctyl ester; unspecified purity) in saline at a dose of 1 mmol/kg bw per day for 2 weeks. The dose was reduced to 0.5 mmol/kg bw per day after 4 days due to overt toxicity. Rats were sacrificed after 14 days, and their livers were dissected for the preparation of peroxisomes and microsomes. β-Oxidation in isolated peroxisomes was increased 2-fold compared with the saline control. Microsomal glutathione reductase activity was increased by approximately 40% over the controls, whereas glutathione peroxidase activity was increased by approximately 30%.

MCPA (unspecified purity) in saline was administered by gavage to groups of 4–7 male Wistar rats at 0 or 0.5 mmol/kg bw per day for 14 days. Rats were killed 24 hours after the last dose. and the kidneys and proximal small intestine were prepared for the analysis of microsomal enzymes. Intestinal AHH and kidney ECOD and EH activities were significantly increased ($P < 0.05$) over the control values (1.7-, 2- and 1.6-fold, respectively). There was no induction of intestinal ECOD, EH or UDPGT (Ahotupa et al., 1983).

Male Han Wistar rats ($n = 4$) were gavaged with MCPA (unspecified purity) at 100 mg/kg bw per day, 5 days/week, for 2 weeks. Control rats ($n = 4$) were dosed with the dimethyl sulfoxide (DMSO) vehicle. There was no effect on body weight gain, relative liver weight or the level of non-protein sulfhydryl groups in the liver. Hepatic peroxisomes were significantly increased ($P < 0.01$) compared with the controls (14.2 /100 μm^2 versus 7.7/100 μm^2, respectively), but there was no increase in peroxisome size. In hepatic microsomes, the activities of cytochrome P450, ECOD and propionaldehyde dehydrogenase were significantly higher ($P < 0.01$ or 0.05) than the control values (1.2-, 1.4- and 1.3-fold increases, respectively), whereas the activity of UDPGT was significantly lower ($P < 0.05$) than the control value (approximately 1.3- to 1.4-fold lower). There was no effect on the activities of reduced nicotinamide adenine dinucleotide phosphate (NADPH)-cytochrome P450 reductase or NADPH diaphorase (Mustonen et al., 1989).

The effects of MCPA on hepatic peroxisomal enzymes were investigated in male Sprague-Dawley rats (five per group) following 2 weeks of daily gavage dosing at 0, 100, 200 or 300 mg/kg bw per day. There was a dose-related reduction in body weight, which was statistically significant ($P < 0.01$ or 0.05) at 200 and 300 mg/kg bw per day (297, 283, 277 and 263 g at 0, 100, 200 and 300 mg/kg bw per day, respectively). Hepatic cholesterol and/or phospholipid levels were significantly lower ($P < 0.01$ or 0.05) than the control values at every dose. Fatty acid β-oxidation was significantly increased at 200 and 300 mg/kg bw per day (2.5- and 3.5-fold higher than the control value, respectively). Significantly increased L-carnitin-dependent acetyltransferase ($P < 0.01$; 2- to 7-fold higher at every dose) and L-carnitin-dependent octanoyl transferase ($P < 0.05$; 3-fold higher than the control value at 300 mg/kg bw per day) also occurred (Inomata et al., 1991a).

Inomata et al. (1991b) further examined the effects of MCPA and MCPA 2-EHE on hepatic peroxisomal enzymes in male Sprague-Dawley rats. MCPA (unspecified purity) or MCPA 2-EHE (purity 96%) in 0.5% w/v carboxymethylcellulose and olive oil, respectively, were administered to groups of five rats by gavage at 0 or 200 mg/kg bw per day for 2 weeks. Body weight and body weight gain (0–2 weeks) were lower than the control values by approximately 12% and 18%, respectively, for MCPA and 36% and 52%, respectively, for MCPA 2-EHE; these differences were statistically significant ($P < 0.01$). Relative liver weight was approximately 10% higher ($P < 0.01$)

than the control value in the MCPA group. The activities of aniline hydroxylase and ECOD in hepatic microsomes were significantly increased ($P < 0.01$ or 0.05) by 15% and 1.5-fold, respectively, over the control with both MCPA and MCPA 2-EHE. MCPA 2-EHE and MCPA significantly increased ($P < 0.01$ or 0.05) microsomal cytochrome P450 activity (approximately 1.5-fold increase over the controls). MCPA also increased cytochrome b_5 activity ($P < 0.05$; 1.2-fold increase) and NADPH cytochrome *c* reductase activity ($P < 0.01$; 1.3-fold increase); NADH-ferricyanide reductase activity was decreased by approximately 12% ($P < 0.01$). There was no effect on aminopyrine *N*-demethylase or UDPGT activities in hepatic microsomes.

The induction of P450-dependent mixed-function oxidase in the livers of male Wistar rats ($n = 6$) was investigated following gavage dosing with MCPA (unspecified purity) in peanut oil at 0 ($n = 15$), 50, 100 or 200 mg/kg bw per day for 3 days. MCPA had no effect on body weight gain or relative liver weight, but increased the microsomal activity of cytochrome P450 (1.6-fold induction over the controls; $P < 0.05$) and specifically the activity of cytochrome P450 4A1 (4.6-fold induction over the controls; $P < 0.001$). Additionally, microsomal ECOD activity was increased by approximately 2-fold ($P < 0.01$) over the controls (Bacher & Gibson, 1988).

To study the effect of MCPA on peroxisome proliferator-activated receptor (PPAR) activation, COS-1 cells transfected with mouse or human PPARα or PPARγ (and the reporter plasmid pHD(x3)-Luc) were incubated with MCPA (unspecified purity) in DMSO at concentrations up to 400 µmol/l. 2,4-D was also tested up to concentrations of 800 µmol/l. At MCPA concentrations of 50 and 400 µmol/l (but not 100 or 200 µmol/l), there was a significant increase ($P < 0.05$) in PPARα activation (2.1- and 1.2-fold higher luciferase activity than the solvent control, respectively). There was no effect on PPARγ activation. No effect was observed for 2,4-D. The authors concluded that MCPA and 2,4-D did not directly transactivate mouse or human PPAR and suggested that metabolism may be required to activate them (Maloney & Waxman, 1999).

(c) Haematology

Pistl et al. (2003) investigated the immunotoxic potential of MCPA (purity 99.1%) (and seven other pesticides) in isolated sheep leukocytes at concentrations of 10^{-1} to 10^{-6} mol/l. Cytotoxicity (measured as a decrease in spontaneous leukocyte migration) occurred at 10^{-1} mol/l (16 cm^2 versus 27.6 cm^2 in the controls; $P < 0.01$). Immunotoxicity (measured as a decrease in lymphocyte activation with phytohaemagglutinin) occurred at concentrations ranging from 10^{-2} to 10^{-6} mol/l ($P < 0.001$). MCPA did not suppress the metabolic activity of sheep phagocytes in the iodonitrotetrazolium reductase test.

MCPA (purity ≥ 99%) (and seven other phenoxyacetic acid herbicides) was tested for its ability to inhibit the aggregation of human platelets in vitro. Platelet aggregation was induced with adenosine diphosphate at 1–32 µmol/l, adrenaline at 0.32–32 µmol/l or collagen at 7.5–30 µg/ml in the presence of MCPA at 0, 0.5, 0.1, 0.5, 1.0 or 2.0 mg/ml. Dose-dependent inhibition of platelet aggregation occurred at MCPA concentrations of 0.1–2.0 mg/ml (Elo et al., 1991).

Bukowska et al. (2000) examined the effect of MCPA sodium salt (purity 98%) (and other phenoxyacetic acid herbicides) on the catalase activity of human erythrocytes in vitro. At concentrations of 100, 500 and 1000 ppm, MCPA had no effect on the activity of standard catalase following incubation for 1 or 3 hours. Following incubation for 24 hours, a slight decrease (approximately 5% compared with the controls; $P < 0.05$) in activity was detected at 1000 ppm only. There was no significant effect of MCPA (100, 500 and 1000 ppm) on the catalase activity of erythrocytes following a 1-hour incubation. Significant ($P < 0.05$) increases (3-hour incubation; < 6% of the control) or decreases (24-hour incubation; < 10% of the control) were considered to be incidental findings due to their inconsistency and small magnitude. In a subsequent study, MCPA (98% purity) at concentrations up to 1000 ppm had no effect on the activity of acetylcholinesterase in human erythrocytes (Bukowska & Hutnik, 2006).

MCPA sodium salt (purity 98%) (and other phenoxyacetic acid herbicides) in phosphate-buffered saline or ethanol, at concentrations up to 4 mmol/l, was tested for its potential to damage human erythrocytes by measuring haemolysis, haemoglobin oxidation and lipid peroxidation. There

was a concentration-related increase in haemolysis that was statistically significant ($P < 0.05$) at 2 and 4 mmol/l (1.1%, 1.2%, 1.8%, 2.6% and 3.5% at 0, 0.5, 1, 2 and 4 mmol/l, respectively). Methaemoglobin formation was also significantly increased ($P < 0.05$) compared with the solvent control (0.8%, 4.1%, 6.1%, 8.7% and 11.7% at 0, 0.5, 1, 2 and 4 mmol/l, respectively). There was also a treatment-related increase in lipid peroxidation that was statistically significant ($P < 0.05$) at and above 1 mmol/l (approximately 0%, 5%, 10%, 40% and 80% at 0, 0.5, 1, 2 and 4 mmol/l, respectively). The manufacturing impurity and bacterial degradant, 4-chloro-2-methylphenol, and the environmental degradant, 2,4-dimethylphenol, were found to cause similar damage to human erythrocytes (albeit a somewhat higher level of haemolysis at 2 and 4 mmol/l) (Duchnowicz, Koter & Duda, 2002).

(d) Novel plant metabolite

4-Chloro-2-carboxyphenoxyacetic acid (CCPA) is a novel plant metabolite of MCPA not identified in animal studies (Figure 3).

Figure 3. Formation of CCPA in plants

From van Ravenzwaay et al. (2005)

CCPA (purity 99.45%) in 0.5% w/v carboxymethylcellulose was administered by gavage as a single dose of 2000 mg/kg bw to five male and five female Crl:CD®(SD)IGS BR rats. There were no deaths, clinical signs or effects on body weight in the 14-day post-dosing observation. The necropsy was unremarkable. The oral LD_{50} was therefore greater than 2000 mg/kg bw (Kern, 1999).

In a bacterial reverse mutation assay using *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* WP2*uvrA*, CCPA (purity 99%) in DMSO was not genotoxic (or cytotoxic) up to concentrations of 5000 µg/plate with and without S9 (Mecchi, 2002).

CCPA (purity 99.7%) was admixed in the diet at a concentration of 0, 2000 or 12 000 ppm and fed to groups of five Wistar rats (CrlGlxBrlHan:WI strain) of each sex for 4 weeks. For comparison, separate groups of rats were concurrently fed a diet containing 2000 ppm MCPA (purity 97%). Mean intakes of CCPA were 0, 176 and 1076 mg/kg bw per day for males and 0, 194 and 1144 mg/kg bw per day for females at 0, 2000 and 12 000 ppm, respectively. The mean intakes of MCPA were 166 mg/kg bw per day for males and 173 mg/kg bw per day for females. Observations for mortality and clinical signs were made daily. Detailed open-field observations were made prior to the commencement of dosing and on a weekly basis thereafter. Body weight and feed and water consumption were recorded weekly. A functional observational battery and motor activity assessment were undertaken after 4 weeks. Blood and urine were collected after 24 or 25 days for the analysis of haematology, clinical chemistry or urine analysis parameters. At the end of the study, all rats were sacrificed and necropsied. Organs were weighed and tissues examined histopathologically. There were no deaths or clinical signs. CCPA had no effect on body weight, body weight gain or feed or water consumption. In contrast, the mean body weight and body weight gain of MCPA-treated rats were significantly lower ($P < 0.01$ or 0.05) than the control values (approximately 17% [day 28] and 37% [days 7–28] lower than the controls, respectively, in males; approximately 10% [day 28] and

31% [days 7–28] lower than the controls, respectively, in females). Feed consumption was up to approximately 23% lower than the control value in MCPA-treated males and up to approximately 16% lower than the control value in females. Feed conversion efficiency was significantly lower than the control value in males on day 7 ($P < 0.01$), day 14 ($P < 0.01$) and day 21 ($P < 0.05$). Water consumption was approximately 40% higher than the control value in MCPA-treated females on day 7 ($P < 0.05$) and day 21 ($P < 0.01$). Functional observational battery and motor activity assessments were unremarkable. There were no treatment-related effects on any haematology parameters.

Selected clinical chemistry and urine analysis parameters are presented in Table 37. At 2000 ppm CCPA, blood glucose was significantly lower ($P < 0.05$) than the control value in males; however, in the absence of a difference at 12 000 ppm, this was not considered treatment related. At 12 000 ppm CCPA, significantly reduced albumin ($P < 0.05$) and increased magnesium levels ($P < 0.05$) occurred in females. In this same group, urine volume was markedly lower than the control value, whereas specific gravity was increased in both sexes, but not significantly. There was a small increase in urobilinogen in the urine of both sexes. The following effects occurred in MCPA-treated rats: significantly elevated ($P < 0.05$) ALT in females (approximately 32% higher than the control value); decreased serum glucose ($P < 0.05$, both sexes), total bilirubin ($P < 0.01$, females) and albumin ($P < 0.05$, females); and increased serum creatinine ($P < 0.05$, males) and magnesium ($P < 0.05$, females). In addition, epithelial casts were detected in the urine of three males.

Table 37. Clinical chemistry and urine analysis findings in rats exposed to CCPA or MCPA for 4 weeks in the diet

Parameter	Mean value							
	Males				Females			
	Control	CCPA 2000 ppm	CCPA 12 000 ppm	MCPA 2000 ppm	Control	CCPA 2000 ppm	CCPA 12 000 ppm	MCPA 2000 ppm
ALT (µkat/l)	0.61	0.54	0.56	0.69	0.50	0.55	0.49	0.66*
Creatinine (µmol/l)	46.2	46.0	47.1	52.5*	47.7	46.3	48.3	47.5
Glucose (mmol/l)	7.92	6.09*	6.68	4.80*	6.34	6.11	5.54	4.81*
Total bilirubin (µmol/l)	2.34	2.16	2.44	1.98	2.40	2.44	2.11	1.34**
Albumin (g/l)	34.81	35.26	35.21	35.08	39.37	38.94	36.95*	36.46*
Magnesium (mmol/l)	0.96	1.00	0.92	0.96	0.93	0.96	1.00*	1.03*
Urine volume (ml)	3.6	3.5	2.4	3.5	2.2	2.0	1.1	1.9
Urine specific gravity (g/l)	1068	1061	1084	1057	1065	1073	1124	1060
Urine urobilinogen								
- normal	3	4	0	0	4	3	1	0*
- > 68 µmol/l	2	1	5	5	1	2	4	5

From Mellert et al. (2004); van Ravenzwaay et al. (2005)
* $P < 0.05$; ** $P < 0.01$

There were no treatment-related macroscopic abnormalities. Because of the lower terminal body weight, the absolute weights of most organs were significantly lower than the control values in males (ranging from approximately 18% lower for the epididymis to approximately 37% for the

thymus), whereas the absolute weights of the thymus, adrenals and ovaries were lower in females (approximately 40%, 29% and 36% lower, respectively). The relative weights of the testes and brain were significantly higher than the control values in males (approximately 14% and 19%, respectively), whereas the relative weights of the ovaries and adrenals of females were significantly lower (approximately 29% and 21% lower, respectively). In CCPA-treated rats, no significant differences in organ weights were determined.

Minimal focal degeneration of seminiferous tubules of the testes was observed in three of five MCPA-treated males, and increased cellularity of bone marrow occurred in two females, consistent with observations from other studies. There was an increase in the severity of renal calcification at the outer/inner medullary transition in high-dose, CCPA-treated females (two females with grade 3 calcification versus zero in the control); however, all females in the control and high-dose CCPA groups had renal calcification.

The NOAEL for CCPA was 2000 ppm (equal to 176 mg/kg bw per day for males and 194 mg/kg bw per for females), based on reduced serum albumin, increased serum magnesium, increased specific gravity and increased renal calcification at 12 000 ppm (equal to 1076 mg/kg bw per day for males and 1144 mg/kg bw per day for females). CCPA was concluded to be less toxic than MCPA (Mellert et al., 2004; van Ravenzwaay et al., 2005).

3. Observations in humans

3.1 Occupational exposure studies

The induction of sister chromatid exchanges (SCEs) was examined in the peripheral lymphocytes of 35 forestry workers exposed to MCPA (500 g/l as an iso-octylester formulation), 2,4-D (550 g/l as an amine salt formulation) or a mixture of both (333 g/l 2,4-D; 167 g/l MCPA). A group of 15 unexposed workers served as the control. Exposure was estimated by analysing the concentration of MCPA and 2,4-D in urine. Three successive blood samples were taken before, during (after 6–26 days of exposure) and after (within 2 days after the cessation of spraying) one 4-month spraying season for the analysis of SCEs. Urinary concentrations of both herbicides were highly variable across the 35 subjects, ranging from 0 to approximately 11 g/l (mean ± 1 standard deviation of 1.80 ± 2.85 g/l). The concentration of MCPA ranged from 0 to 1.14 mg/l. In 7 of 19 subjects, MCPA and 2,4-D were not detectable. There was no significant difference in the frequency of SCEs in the exposed versus the non-exposed subjects (Linnainmaa, 1983).

The effects of short-term exposure (1–3 days) to commercial formulations containing a mixture of MCPA and 2,4-D on peripheral blood lymphocytes were assessed in 10 male farmers. Blood was sampled within 1–7 days before and 1–12 days and 50–70 days after exposure. Lymphocyte subsets were quantified, and mononuclear cells were separated and assayed for natural killer (NK) cell function and mitogen proliferative response. During the first sampling period, the proportions of several T cell subtypes (T helper, suppressor T, cytotoxic T, CD8-DR, NK cells) were significantly lower ($P < 0.01$ or 0.05) than those measured prior to exposure. However, the magnitude of these differences was relatively small (< 10%), and there was no difference in total white blood cell or lymphocyte counts. Given the small sample size, variability in the potential sampling window, the absence of a concurrent unexposed group and the lack of analysis of urine or blood levels of MCPA or 2,4-D (to confirm systemic exposure), the study is considered to be preliminary in nature (Faustini et al., 1996).

Coggon et al. (1986) examined the mortality of 5754 workers involved in manufacturing and spraying of MCPA and other phenoxyacetic acid herbicides from 1947 to 1975 in England. Overall mortality (including that from cancer, heart disease and respiratory disease) was less than that of the national population. One soft tissue sarcoma was detected against the expected 0.6. The authors concluded that any risk of soft tissue sarcoma due to MCPA is less than suggested by earlier studies of 2,4,5-T and 2,4,5-trichlorophenol production and must be small in absolute terms.

A study of cancer incidences among 4459 Danish workers involved in the production of MCPA and other phenoxyacetic acid herbicides was undertaken by Lynge et al. (1985). The overall

cancer incidence among workers was unremarkable, with the exception that 5 soft tissue sarcomas were detected in males versus an expected 1.84 for the Danish population. The 5 soft tissue sarcomas were all different tumour types, and only one case was linked directly to manufacturing. The study did not differentiate between exposure to different pesticides and exposure to precursor chemicals involved in their production.

Wiklund & Holm (1986) investigated the risk of soft tissue sarcoma in 354 620 Swedish male agricultural or forestry workers (identified in the 1960 census) due to exposure to phenoxyacetic acid herbicides. A reference cohort of 1 725 845 Swedish men involved in other industries was used as the comparator. In total, 331 cases of soft tissue sarcoma were detected versus 1508 in the reference cohort (relative risk = 0.9; 95% confidence interval [CI] = 0.8–1.0), which indicated that there was no increase in cancer risk. In a follow-up study by Wiklund et al. (1987), the risk of Hodgkin disease and non-Hodgkin lymphoma in a cohort of 20 245 Swedish pesticide applicators was studied. Seventy-two per cent were estimated to have used phenoxyacetic acid herbicides. Eleven cases of Hodgkin disease (versus an expected 9.1 in the general population; rate ratio = 1.20, 95% CI = 0.6–2.16) and 21 cases of non-Hodgkin lymphoma (versus an expected 20.8 in the general population; rate ratio = 1.01, 95% CI = 0.63–1.54) were detected, with no significant increase in the risk of either malignant lymphoma.

Based on a preliminary finding that the standardized mortality ratio (SMR) for cancers of the biliary tract in Japan was associated with rice production, Yamamoto et al. (1986) examined the association (between 1962 and 1975) with environmental contamination with agricultural chemical products. While the analysis detected statistically significant ecological correlations (predominantly in men) between environmental contamination with MCPA and biliary tract cancer (correlation coefficient of 0.26–0.38), the study is considered to have too many confounding factors to allow a meaningful interpretation.

A case–control study involving 30 males with soft tissue sarcoma and 52 males with malignant lymphoma was undertaken by Smith & Christophers (1992) to test whether there was an association with past exposure to phenoxyacetic acid herbicides or chlorophenols. Exposure was assessed by way of a personal interview; 43 men were definitely or probably exposed and 17 possibly exposed for at least 1 day prior to 5 years previously (exposure ranged from 1 hour to 122 weeks). No significant association was found between exposure to phenoxyacetic acid herbicides or chlorophenols and soft tissue sarcoma or malignant lymphoma.

Using an international register maintained by the International Agency for Research on Cancer of 21 863 workers exposed to phenoxyacetic acid herbicides, Kogevinas et al. (1995) reported an increased risk of soft tissue sarcoma in workers exposed to any phenoxyacetic acid herbicide (odds ratio [OR] = 10.3; 95% CI = 1.2–91) or to MCPA or 4-(4-chloro-*o*-tolyloxy)butyric acid (MCPB) (OR = 11.3; 95% CI = 1.30–97.92). No significant association was determined for non-Hodgkin lymphoma. Exposure to chlorophenols and to other raw materials and process chemicals was not associated with an increased cancer risk. The authors concluded that workers with substantial exposure to phenoxyacetic acid herbicides and their contaminants were at higher risk for soft tissue sarcoma, and the risk was not specifically associated with herbicides contaminated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD).

In a follow-up analysis, Kogevinas et al. (1997) examined the same register of 21 863 workers using a more refined estimate of exposure to TCDD and higher chlorinated dioxins, verified in some cases by analysis of dioxins in serum and adipose tissue. An SMR of 2.03 (95% CI = 0.75–4.43) was calculated for workers exposed to phenoxyacetic acid herbicides contaminated with TCDD and higher chlorinated dioxins, which was higher than that expected from national mortality rates. Mortality from all malignant neoplasms (SMR = 1.12, 95% CI = 1.04–1.21), non-Hodgkin lymphoma (SMR = 1.39, 95% CI = 0.89–2.06) and lung cancer (SMR = 1.12, 95% CI = 0.98–1.28) was only marginally higher than expected from national mortality rates. No increased risk of mortality from total neoplasms, non-Hodgkin lymphoma or lung cancer was determined in workers exposed to phenoxyacetic acid herbicides with minimal or no contamination by TCDD and higher chlorinated dioxins, whereas mortality from soft tissue sarcoma was marginally elevated (SMR = 1.35). The

authors concluded that “exposure to herbicides containing TCDD and higher chlorinated dioxins may be associated with a small increase in overall cancer risk and in risk for specific cancers”.

Vena et al. (1998) analysed non-cancer mortality in the same cohort of pesticide workers as studied by Kogevinas et al. (1995, 1997). SMR analysis did not suggest an increase in non-cancer mortality due to exposure to TCDD and higher chlorinated dioxins. However, Poisson regression analyses suggested an increased risk of ischaemic heart disease (rate ratio = 1.67, 95% CI = 1.23–2.26) and diabetes (rate ratio = 2.25, 95% CI = 0.53–9.50) among TCDD-exposed workers.

In a Swedish population-based, case–control study, the relationship between pesticide exposure and non-Hodgkin lymphoma was examined. Exposure data were obtained from 404 individuals with non-Hodgkin lymphoma (diagnosed from 1987 to 1990) and 741 controls via questionnaires answered by the subjects or next-of-kin (in the case of death). An increased risk for non-Hodgkin lymphoma was calculated for exposure to herbicides (OR = 1.6, 95% CI = 1.0–2.5), phenoxyacetic acid herbicides (OR = 1.5, 95% CI = 0.9–2.4) and MCPA (univariate analysis: OR = 2.7, 95% CI = 1.0–6.9; multivariate analysis: OR = 1.3, 95% CI = 0.4–3.9). For herbicides in total (and for phenoxyacetic acids), the highest risks were calculated when exposure first occurred 10–20 years before diagnosis. In contrast, for MCPA, only exposure during the most recent decade (i.e. 1–10 years) before diagnosis was associated with an increased risk of non-Hodgkin lymphoma (Hardell & Eriksson, 1999).

A case–control study of 910 Swedish patients with non-Hodgkin lymphoma was conducted on cases diagnosed between December 1999 and April 2002. Controls (1016) were chosen randomly from a national population registry. Exposure was evaluated using a questionnaire, with the minimum criterion for inclusion of a single full day. Of the phenoxyacetic acid herbicides, the highest OR (2.81) was calculated for MCPA (95% CI = 1.27–6.22), with all cases having a latency period of greater than 10 years. The OR was 3.76 for less than 32 days of exposure and 1.66 for greater than 32 days of exposure. MCPA appeared particularly associated with histological diffuse large B cell lymphoma (OR = 3.91, 95% CI = 1.48–10.5) or unspecified non-Hodgkin lymphoma (OR = 9.31, 95% CI = 2.11–41.2). The authors concluded that the study confirms an association between exposure to phenoxyacetic acid herbicides and non-Hodgkin lymphoma (Eriksson et al., 2008).

3.2 Poisoning case reports

A 32-year-old Indian male, with a history of tuberculosis and psychosis, was discovered by relatives in a deep coma and unresponsive to painful stimuli. His pupils were small and unreactive and without gross hypertonia; he showed twitching of the face followed by clonic-tonic convulsions. Pulse and respiration were rapid. Liver function test and plasma electrolytes were reportedly within the normal range. Blood pressure declined, and the man died approximately 20 hours after admission to hospital. Based on information provided by relatives, it was estimated that the man ingested 85 ml of a formulation (Verdone) that contained MCPA (equivalent to approximately 22 g MCPA). Autopsy failed to establish the cause of death. Other than evidence of tuberculosis, there were no histopathological abnormalities. MCPA was detected in the gastric washing (0.3 g), liver (0.158 g), urine (0.097 g), stomach (0.041 g), brain (0.038 g), heart (0.037 g) and blood (23 mg/100 ml) (Popham & Davies, 1964).

A 75-year-old retired blacksmith, with a history of congestive cardiac failure and depression, was discovered unconscious near an empty 57 ml bottle of a formulation (Verdone) that contained 25% MCPA. He was admitted to the hospital in a coma and was unresponsive to all stimuli. Blood pressure decreased continuously, and he remained deeply unconscious until death approximately 21 hours after admission (Johnson & Koumides, 1965).

A 61-year-old man, with a recent history of depression, ingested an unknown volume of MCPA. Shortly afterwards, he vomited, his speech became slurred, his face twitched and his limbs jerked. The man was unconscious on arrival at hospital; breathing was laboured, pupils were unresponsive to light and general twitching of the muscles and clonic spasms of the limbs were noted (without major convulsions). Occult blood was observed in the faeces for 21 days. In addition to these central nervous system abnormalities, elevated ALT, ALP and blood urea nitrogen, decreased

haemoglobin and platelets, proteinuria and glycosuria occurred. Serum electrolytes were normal. Hypostatic pneumonia developed. The man recovered completely after prolonged and intensive therapy, including endotracheal intubation, tracheostomy, alkaline diuresis and supportive therapy (Jones et al., 1967).

Roberts et al. (2005) evaluated the toxicity and clinical management of acute MCPA poisoning in a prospective study of 181 self-poisoning cases in Sri Lanka. Patient history, clinical signs and blood (91 patients) were obtained on admission to hospital. Creatine kinase activity was measured in 10 patients who tested positive to MCPA on admission (6 patients had MCPA concentrations in blood > 300 mg/kg, the remainder < 300 mg/kg; limit of detection = 0.05 mg/kg). Minimal toxicity was predominantly observed (85% of patients), manifesting as spontaneous vomiting (44% of patients), with more severe clinical signs (rhabdomyolysis, renal dysfunction and coma) appearing uncommon. Eight patients (4.4%) died, seven within 24–48 hours of poisoning due to cardiorespiratory arrest of an unclear etiology. A delay in the time to hospital admission did not appear to contribute to this outcome. Oliguria or dark-coloured urine was observed in five patients, suggesting renal toxicity. Clinical signs and elevated serum creatine kinase were poorly correlated with plasma concentrations of MCPA. Recommended interventions were limited to correcting acidosis and maintaining adequate urine output.

In a case of fatal poisoning, the highest concentration of MCPA was detected in the stomach contents (10 200 µg/g), the right lung (1362 µg/g), liver (1135 µg/g), heart blood (888 µg/g), brain (771 µg/g), right kidney (756 µg/g) and urine (52 µg/g). 4-Chloro-2-methylphenol was detected as a possible metabolite in body fluids and tissues (Takayasu et al., 2008).

Comments

Biochemical aspects

In studies conducted in rats, dogs and humans using either radiolabelled or unlabelled MCPA, MCPA 2-EHE or MCPA DMA, the time to reach the maximum plasma concentration of radioactivity or of MCPA ion ranged from 1 to 8 hours, depending on the dose. In rats, gastrointestinal absorption was at least 95% of the administered dose, with in vitro data suggesting both a saturable carrier-mediated process and a non-saturable process involving simple diffusion. Following oral dosing of rats, there was no evidence of accumulation of radioactivity in any tissues, with the concentrations in the majority of tissues lower than those in blood. In a study conducted in pregnant rats, the concentration of radioactivity in fetal plasma and amniotic fluid was approximately 3- to 9-fold lower than that in maternal plasma.

MCPA, like other organic acids, is excreted via the kidneys by an active mechanism, with this process saturated at sufficiently high doses in rats, dogs and humans; the threshold of renal saturation is lower in dogs than in rats and humans. In rats, excretion of radioactivity was predominantly via the urine (approximately 90% of the administered dose), with relatively low levels detected in faeces (approximately 5% of the administered dose), although increasing the dose from 5 to 100 mg/kg bw increased the level of radioactivity in the faeces of females (approximately 20% of the administered dose). A different pattern of excretion was evident in dogs, with a greater proportion of radioactivity detected in faeces (17% of the administered dose at 5 mg/kg bw and 49% at 100 mg/kg bw). The plasma elimination half-life was longer in dogs (approximately 45 hours) than in rats and humans (approximately 6–10 hours), with this slower elimination resulting in higher systemic exposure at comparable doses. As a consequence of its longer residence time, MCPA ion undergoes more extensive metabolism in dogs. MCPA ion was the predominant compound detected in rat and dog excreta, followed by HMCPA and glycine-conjugated MCPA. The proportions of MCPA and HMCPA in rat urine ranged from about 51% to 80% and from about 6% to 16% of the administered dose, respectively. In rat faeces, MCPA and HMCPA accounted for approximately 1–2% and 1–7% of the administered dose, respectively. In dogs, MCPA (up to approximately 30% of the administered dose) and HMCPA (up to approximately 15% of the administered dose) were detected in urine, in addition to glycine and taurine conjugates (up to 38% and 10% of the administered dose,

respectively). In dog faeces, MCPA and the glycine and taurine conjugates were identified (up to 28%, 4% and 19% of the administered dose, respectively).

MCPA induced a variety of drug metabolizing enzymes in rats at relatively high oral doses (100–300 mg/kg bw) and increased fatty acid β-oxidation and the number of hepatic peroxisomes. MCPA's peroxisome proliferating potential was not overtly expressed at lower doses in rodents, with a decrease in serum triglycerides the most consistent finding.

Toxicological data

Acute toxicity studies were conducted with MCPA, MCPA 2-EHE and MCPA DMA. The oral LD_{50} in rats was 500–1200 mg/kg bw, depending on the vehicle. Clinical signs generally occurred within hours of dosing and included piloerection, apathy, hunched posture, abnormal gait, decreased respiration, ptosis, pallor and occasionally ataxia, twitching and tonic convulsions; survivors recovered within about 2 days. The dermal LD_{50} in rats and rabbits was greater than 2000 mg/kg bw, whereas the LC_{50} in rats was greater than 4.5 mg/l. Nil to slight skin irritation and slight to severe eye irritation occurred in rabbits, depending on the formulation. No skin sensitization occurred in guinea-pigs (maximization, open epicutaneous and Buehler tests).

The target organs for MCPA ion are the kidney, liver and blood. In laboratory animals, toxicity following repeated dosing typically manifested as perturbations in clinical pathology parameters (increased serum creatinine, urea, liver enzymes and clotting time and reduced red cell parameters and serum protein) and, in some species, increased kidney and liver weights in conjunction with histopathological changes in these organs. Reduced body weight gain and feed consumption, clinical signs and deaths also occurred.

Short-term studies of toxicity of less than 12 months' duration using MCPA, MCPA 2-EHE or MCPA DMA were performed in rats and dogs. In a 3-month study in rats, the NOAEL was 150 ppm (equal to 12 mg/kg bw per day) for increased serum creatinine and kidney weight at 450 ppm (equal to 35 mg/kg bw per day). In dogs, the overall NOAEL from five studies of 13 or 52 weeks' duration was 6 ppm (equal to 0.2 mg/kg bw per day) for increased serum creatinine and urea and increased pigmentation in the proximal tubules at 30 ppm (equal to 1 mg/kg bw per day).

Long-term studies of the toxicity and carcinogenicity of MCPA were conducted in mice and rats, with no indication of any treatment-related neoplastic lesions up to dietary concentrations of 500 ppm in mice and 320 ppm in rats (equal to 83 and 19 mg/kg bw per day, respectively). The chronic NOAEL in mice was 100 ppm (equal to 16 mg/kg bw per day) for increased kidney weight and an increased incidence of intratubular calcification, hyaline casts and tubular epithelial hyperplasia at 500 ppm (equal to 83 mg/kg bw per day). The chronic NOAEL in rats was 80 ppm (equal to 5 mg/kg bw per day), based on increased kidney weight and slight increases in the severity of chronic progressive nephropathy in males and haemosiderin deposition in the spleen of both sexes at 12 but not 24 months at 320 ppm (equal to 19 mg/kg bw per day), findings considered to be of questionable relevance.

The Meeting concluded that MCPA is not carcinogenic in mice or rats.

The genotoxicity of MCPA, MCPA 2-EHE and MCPA DMA has been extensively tested in vitro and in vivo, and all were found to have no genotoxic potential. The Meeting concluded that the MCPA ion is unlikely to be genotoxic to humans.

In the absence of genotoxicity potential and a carcinogenic response in mice and rats, the Meeting concluded that MCPA ion is unlikely to pose a carcinogenic risk to humans.

In multigeneration studies in rats on MCPA or MCPA 2-EHE, there was no evidence of reproductive toxicity up to the highest tested dietary concentrations of 450 and 1600 ppm (equal to 40 and 150 mg/kg bw per day, respectively) over one or two generations. In the two-generation study on MCPA, the NOAEL for parental and offspring toxicity was 150 ppm (equal to 12 mg/kg bw per day) for reduced body weight gain at 450 ppm (equal to 40 mg/kg bw per day). The LOAEL for parental toxicity in this study is consistent with those in four subchronic rat studies. In one-generation studies on MCPA or MCPA 2-EHE, reduced parental body weight gain occurred at the lowest tested dietary

concentrations of 450 and 700 ppm (equal to 40 and 65 mg/kg bw per day, respectively), whereas the NOAEL for offspring toxicity was 750 or 1200 ppm (equal to 115 and 90 mg/kg bw per day, respectively) for reduced body weight gain at 1000 or 1600 ppm (equal to 160 and 230 mg/kg bw per day, respectively).

In rat developmental toxicity studies conducted on MCPA, MCPA 2-EHE and MCPA DMA, the overall NOAEL for maternal toxicity and fetal and embryo toxicity was 60 mg/kg bw per day for reduced maternal body weight gain and feed consumption, reduced fetal body weight and an increase in fetal anomalies (mainly delayed ossification), post-implantation losses and early resorptions at 120 mg/kg bw per day. The reduction in maternal body weight gain and feed consumption was evident 1 or 2 days after the commencement of dosing and persisted throughout gestation. The Meeting noted that the developmental findings occurred only at maternally toxic doses.

In a rabbit developmental toxicity study on MCPA, no developmental effects occurred up to the highest tested dose of 60 mg/kg bw per day. The NOAEL for maternal toxicity was 15 mg/kg bw per day, based on clinical signs (piloerection, no defecation and blood in the bedding) and deaths at 30 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 60 mg/kg bw per day, the highest dose tested.

The Meeting concluded that MCPA ion is not teratogenic in rats or rabbits.

Acute and subchronic neurotoxicity studies were conducted on MCPA, MCPA 2-EHE and MCPA DMA in rats. In the acute studies, clinical signs of toxicity observed 24 hours after dosing and transiently impaired motor activity were attributable to acute systemic toxicity rather than to a direct neurotoxic effect. No pathology of the brain or nervous tissue was observed in these or other toxicity studies. The acute NOAEL for MCPA was 150 mg/kg bw, whereas clinical signs (mainly ataxia) were observed at the lowest tested doses of MCPA 2-EHE and MCPA DMA (160 and 143 mg/kg bw, respectively). The overall NOAEL in the three subchronic studies was 4 mg/kg bw per day, with increased serum creatinine the most consistent effect seen at the next higher dose of 40 mg/kg bw per day.

CCPA is a metabolite of MCPA specific to plants. The oral LD_{50} in rats was greater than 2000 mg/kg bw. CCPA was not mutagenic. In a 4-week dietary study, CCPA was approximately 5-fold less toxic than MCPA. The NOAEL for CCPA was 176 mg/kg bw per day, based on reduced serum albumin, increased serum magnesium, increased urine specific gravity and increased renal calcification at approximately 1100 mg/kg bw per day. The LOAEL for MCPA was approximately 170 mg/kg bw per day. The Meeting concluded that CCPA was less toxic than MCPA ion.

Poisoning case reports described clinical observations in humans following deliberate ingestion of relatively large doses of MCPA formulations. Clinical signs consistent with those observed in laboratory animals and renal toxicity were reported.

The Meeting concluded that the database on MCPA ion was adequate to characterize the potential hazards to fetuses, infants and children.

Toxicological evaluation

The Meeting concluded that the dog was an unsuitable surrogate for humans because of its relatively low renal capacity to excrete MCPA ion, leading to higher toxicity than in other species. Therefore, the Meeting established an acceptable daily intake (ADI) of 0–0.1 mg/kg bw per day for MCPA ion, based on the overall NOAEL of 12 mg/kg bw per day from four subchronic studies in rats for changes in clinical chemistry parameters indicative of effects on the kidneys at 35 mg/kg bw per day and using a 100-fold safety factor. This overall NOAEL is supported by the NOAEL of 12 mg/kg bw per day for parental and offspring toxicity from the two-generation reproductive toxicity study in rats and the NOAEL of 15 mg/kg bw per day for maternal toxicity in the developmental toxicity study in rabbits. The Meeting considered that this ADI would adequately cover the kidney and spleen effects observed in the 2-year rat study at 19 mg/kg bw per day. The ADI is established for the sum of MCPA and its salts and esters, expressed as MCPA acid equivalents.

The Meeting established an acute reference dose (ARfD) of 0.6 mg/kg bw for MCPA ion, based on the overall NOAEL for maternal and developmental toxicity of 60 mg/kg bw in rats and using a 100-fold safety factor. At 120 mg/kg bw, maternal body weight gain and feed consumption were reduced within 1 or 2 days after commencement of dosing in three rat developmental toxicity studies, in addition to an increase in early resorptions in two of these studies. The Meeting considered that the maternal toxicity observed in the rabbit developmental toxicity study was an unsuitable basis for the ARfD because it was not an acute effect. The ARfD is established for the sum of MCPA and its salts and esters, expressed as MCPA acid equivalents.

Levels relevant to risk assessment based on studies conducted on MCPA, MCPA 2-EHE and MCPA DMA

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year study of toxicity and carcinogenicity[a]	Toxicity	100 ppm, equal to 16 mg/kg bw per day	500 ppm, equal to 83 mg/kg bw per day
		Carcinogenicity	500 ppm, equal to 83 mg/kg bw per day[b]	—
Rat	Thirteen-week studies of toxicity[a,c]	Toxicity	150 ppm, equal to 12 mg/kg bw per day	450 ppm, equal to 35 mg/kg bw per day
	Two-generation study of reproductive toxicity[a]	Reproductive toxicity	450 ppm, equal to 40 mg/kg bw per day[b]	—
		Parental toxicity	150 ppm, equal to 12 mg/kg bw per day	450 ppm, equal to 40 mg/kg bw per day
		Offspring toxicity	150 ppm, equal to 12 mg/kg bw per day	450 ppm, equal to 40 mg/kg bw per day
	Developmental toxicity studies[c,d]	Maternal toxicity	60 mg/kg bw per day	120 mg/kg bw per day
		Embryo and fetal toxicity	60 mg/kg bw per day	120 mg/kg bw per day
Rabbit	Developmental toxicity study[d]	Maternal toxicity	15 mg/kg bw per day	30 mg/kg bw per day
		Embryo and fetal toxicity	60 mg/kg bw per day[b]	—

[a] Dietary administration.
[b] Highest dose tested.
[c] Two or more studies combined.
[d] Gavage administration.

Estimate of acceptable daily intake for humans

0–0.1 mg/kg bw

Estimate of acute reference dose

0.6 mg/kg bw

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposure

Critical end-points for setting guidance values for exposure to MCPA ion

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid and almost complete
Distribution	Widespread tissue distribution
Potential for accumulation	No potential for accumulation
Rate and extent of excretion	Rapid except in dogs
Metabolism in animals	Limited; more extensive in dogs
Toxicologically significant compounds in animals, plants and the environment	MCPA, CCPA (plant metabolite)
Acute toxicity	
Rat, LD_{50}, oral	> 500 mg/kg bw
Rat, LD_{50}, dermal	> 2000 mg/kg bw
Rat, LC_{50}, inhalation	> 4.5 mg/l (4 h, whole body)
Rabbit, dermal irritation	Non-irritating to slightly irritating
Rabbit, ocular irritation	Slightly to severely irritating
Dermal sensitization	Non-sensitizing (guinea-pigs)
Short-term studies of toxicity	
Target/critical effect	Kidney, liver and blood
Lowest relevant oral NOAEL	12 mg/kg bw per day (rat)
Lowest relevant dermal NOAEL	160 mg/kg bw per day (rat)
Lowest relevant inhalation NOAEC	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Kidney, liver and blood
Lowest relevant NOAEL	16 mg/kg bw per day (mouse)
Carcinogenicity	Not carcinogenic
Genotoxicity	
	Not genotoxic
Reproductive toxicity	
Target/critical effect	No evidence of reproductive toxicity (rat)
Lowest relevant parental NOAEL	12 mg/kg bw per day
Lowest relevant offspring NOAEL	12 mg/kg bw per day
Lowest relevant reproductive NOAEL	40 mg/kg bw per day (highest dose tested)
Developmental toxicity	
Target/critical effect	Effects on fetuses at maternally toxic doses (rat)
Lowest relevant maternal NOAEL	15 mg/kg bw per day (rabbit)
Lowest relevant embryo/fetal NOAEL	60 mg/kg bw per day (rat and rabbit)
Neurotoxicity	
Acute and subchronic neurotoxicity	Not neurotoxic

Medical data	
	Effects following human poisonings consistent with laboratory animal findings

Summary

	Value	Studies	Safety factor
ADI	0–0.1 mg/kg bw	Short-term repeated-dose studies (rat), two-generation reproductive toxicity study (rat) and developmental toxicity study (rabbit)	100
ARfD	0.6 mg/kg bw	Developmental toxicity studies (rat)	100

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PICOXYSTROBIN

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Explanation

Picoxystrobin is the International Organization for Standardization (ISO)–approved name for methyl (*E*)-3-methoxy-2-[2-(6-trifluoromethyl-2-pyridyloxymethyl)phenyl]acrylate (International Union of Pure and Applied Chemistry) (Chemical Abstracts Service No. 117428-22-5). Picoxystrobin is a broad-spectrum, systemic cereal fungicide from the strobilurin group. It is active against plant pathogens from the four major groups of plant pathogenic fungi—namely, Deuteromycetes, Basidiomycetes, Ascomycetes and Oomycetes. Picoxystrobin's mode of fungicidal activity is to block mitochondrial electron transport at the Q_o site of complex III, reducing adenosine triphosphate (ATP) production and inhibiting cellular respiration. Picoxystrobin has been known under the development codes ZA1963 and DPX-YT669. Initial production batches had a purity of approximately 93%; current production batches have a purity of greater than 99%.

Picoxystrobin has not been evaluated previously by the Joint FAO/WHO Meeting on Pesticide Residues and was reviewed at the present meeting at the request of the Codex Committee on Pesticide Residues.

All critical studies contained statements of compliance with good laboratory practice and met the minimum requirements of the applicable Organisation for Economic Co-operation and Development (OECD) or national test guidelines.

Evaluation for acceptable daily intake

1. Biochemical aspects

The structure of and position of radiolabels in picoxystrobin used in the absorption, distribution, excretion and metabolism studies are illustrated in Figure 1.

Figure 1. Structure of picoxystrobin and position of radiolabels

F F N O 2* F *1 O O

1* (^{14}C-pyridyl)-labelled picoxystrobin

2* (^{14}C-phenyl)-labelled picoxystrobin

1.1 Absorption, distribution and excretion

(a) Oral route

Rats

In an initial study, two groups of Alpk:AP$_f$SD rats, each comprising one male and one female, were administered (^{14}C-pyridyl)- or (^{14}C-phenyl)-labelled picoxystrobin (> 97% radiochemical purity as a single oral dose of 10 mg/kg body weight [bw]) in polyethylene glycol (PEG) 600. The excretion of radioactivity was monitored in urine, faeces and exhaled air. Twenty-four hours after dosing, the rats were killed to investigate the distribution of radioactivity using whole-body autoradiography. Urine and faeces were frozen by collection over solid carbon dioxide, and expired air was monitored for radiolabelled carbon dioxide and other volatile metabolites for 24 hours after dosing. At the end of the collection period, rats were killed and frozen rapidly. The frozen carcasses were processed for autoradiography. The amounts of radioactivity in the dose preparation, urine, plasma, faeces and expired air were measured.

The whole-body autoradiograms showed no marked differences in the distribution of radioactivity between male and female rats dosed with either radiolabelled form of picoxystrobin. In all rats, the greatest intensity of labelling was present in the gastrointestinal tract contents. The absorbed radioactivity was predominant in the liver, with lower intensities apparent in the kidneys, associated with urinary excretion of metabolites. A low intensity of radioactivity was apparent in the blood; consequently, low levels of radiolabel were evident in many tissues, including the heart, lungs and nasal passages. Negligible proportions (< 1%) of the dose were metabolized to volatile exhaled metabolites (Davies, 1997).

The excretion and distribution of picoxystrobin (purity 99.8%; radiochemical purity > 98%; specific activity 2 GBq/mmol) in rats were investigated following a single oral administration of a low dose of 10 mg/kg bw or a high dose of 100 mg/kg bw. Five male and five female Alpk:AP$_f$SD rats

received a single oral dose of (^{14}C-phenyl)-labelled picoxystrobin in PEG 600. Urine was collected 6 hours after dosing, and urine and faeces were collected after 12, 24, 36, 72, 96 and 120 hours. The study was terminated 5 days after dosing, when representative samples of blood, tissues and the gastrointestinal tract contents were removed and submitted to radiochemical analysis.

Following administration of a low dose of 10 mg/kg bw, excretion in both sexes was extensive (Table 1). The majority of urinary excretion occurred within 24 hours. At study termination, all tissue concentrations were low. With the exception of the liver, kidneys, gastrointestinal tract, blood and bone (males only), tissue concentrations of picoxystrobin were less than 0.1 µg equivalents (Eq) per microgram. The amount of administered radioactivity present in the tissue and residual carcass was less than 1%. At the high dose of 100 mg/kg bw, excretion of administered radioactivity was extensive (Table 1) but slightly delayed compared with the low dose. At study termination, the amount of radioactivity present in the tissues and residual carcass of both sexes was low, with the exception of liver, kidneys, gastrointestinal tract, blood and plasma; all tissue concentrations were below 1 µg Eq/g. Following administration of 14 consecutive daily oral doses of unlabelled picoxystrobin at 10 mg/kg bw per day and a 10 mg/kg bw dose of (^{14}C-phenyl)-labelled picoxystrobin, the pattern of distribution and excretion was similar to that seen with a single 10 mg/kg bw dose. With all three dose levels, females excreted a greater proportion of the dose in urine than did males (Table 1).

Table 1. Mean percentage recoveries of administered radioactivity 120 hours after a single oral dose or repeated oral doses of (^{14}C-phenyl)-labelled picoxystrobin (mean of five rats)

	% of administered dose					
	Males			Females		
	10 mg/kg bw	100 mg/kg bw	10 mg/kg bw (repeated)	10 mg/kg bw	100 mg/kg bw	10 mg/kg bw (repeated)
Urine	21	18	19	34	26	32
Faeces	78	74	77	61	65	63
Gastrointestinal tract contents	0.4	0.6	0.3	0.8	0.8	0.5
Cage wash	0.5	0.4	0.5	0.8	1	0.9
Tissues, including carcass	0.8	0.8	0.7	0.9	0.9	0.8
Total	**100**	**94**	**98**	**97**	**94**	**97**

From Brown (1998a,b,c)

The biliary excretion of picoxystrobin (purity 99%) was assessed in Alpk:AP$_f$SD bile duct–cannulated rats receiving a single oral dose of (^{14}C-pyridinyl)-labelled picoxystrobin (radiochemical purity 97%; specific activity 1.9 GBq/mmol) or (^{14}C-phenyl)-labelled picoxystrobin (radiochemical purity 97%; specific activity 1.9 GBq/mmol) at a dose of 100 mg/kg bw in PEG 600 (two males and two females for each radiolabel). Urine was collected at 6 hours and urine and faeces were collected at 12, 24, 36 and 48 hours after dosing with radiolabelled material; bile was collected at 2, 4, 6, 8, 12, 24, 36 and 48 hours.

Following a single oral dose of (^{14}C-phenyl)-labelled picoxystrobin at 100 mg/kg bw, males excreted 2% of the dose in the urine, 71% via bile and 31% in faeces. Females excreted 24% in urine, 45% via bile and 20% in faeces. Following a single oral dose of (^{14}C-pyridinyl)-labelled picoxystrobin at 100 mg/kg bw, males excreted 4.5%, 72% and 18% and females excreted 17%, 66% and 21% in the urine, bile and faeces, respectively. These data, together with tissue and cage wash residues, indicate that oral absorption of picoxystrobin at a dose of 100 mg/kg bw was 75% or greater, with the greatest proportion being eliminated via the bile and subsequently being excreted in the faeces. The

reduction in urinary excretion indicates that a degree of enterohepatic recirculation occurs in non-cannulated animals (Macpherson, 1999).

The pharmacokinetics of [^{14}C]picoxystrobin (purity 99.9%; lot DPX-YT 669-003) was investigated in the plasma and red blood cells of male and female Sprague-Dawley Crl:CD(SD) rats (four rats of each sex per dose per label). [Phenyl(U)-^{14}C]picoxystrobin or [pyridine-3-^{14}C]picoxystrobin at a dose of 10 or 100 mg/kg bw were administered by gavage in PEG 400 vehicle (4 ml/kg bw). Whole blood was collected predosing and at 15 and 30 minutes and 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144 and 168 hours after dose administration. The concentrations of ^{14}C residues were quantified in plasma by liquid scintillation counting (LSC) and in red blood cells by combustion and LSC.

Results (Table 2) were broadly independent of sex and radiolabel position. At the low dose, absorption was relatively rapid (peak concentration in plasma [C_{max}] typically reached before 4 hours [T_{max}]), but delayed at the high dose level (C_{max} reached in approximately 12 hours). Area under the plasma concentration–time curve (AUC) values showed a less than proportionate increase with dose, indicating that absorption was approaching saturation at 100 mg/kg bw relative to the low dose.

Table 2. Mean plasma kinetic parameters in rats administered [^{14}C]picoxystrobin

	10 mg/kg bw		100 mg/kg bw	
	Phenyl[U]-^{14}C	Pyridine-3-^{14}C	Phenyl[U]-^{14}C	Pyridine-3-^{14}C
Males				
C_{max} (µg Eq/g)	4.8	3.4	12.4	14.8
T_{max} (h)	2.2	3.0	12	12
$AUC_{0-\infty}$ (µg·h/g)	110	102	605	579
Elimination $t_{½}$ (h)	47	33	37	46
Females				
C_{max} (µg Eq/g)	2.8	4.5	18	11
T_{max} (h)	7[a]	0.6	9.3	12
$AUC_{0-\infty}$ (µg·h/g)	86	87	710	453
Elimination $t_{½}$ (h)	40	34	31	33

From Himmelstein (2010)

AUC, area under the plasma concentration–time curve; C_{max}, peak concentration in plasma; Eq, equivalent; $t_{½}$, half-life; T_{max}, time to C_{max}

[a] Very variable; standard deviation = 5.9 hours.

The tissue distribution of [^{14}C]picoxystrobin (purity 99.9%; lot DPX-YT 669-003) was investigated in male and female Sprague-Dawley Crl:CD(SD) rats (four rats of each sex per dose per label). Doses of 10 or 100 mg/kg bw of a 1:1 mixture of [phenyl(U)-^{14}C]picoxystrobin (radiochemical purity 97%; specific activity 1.9 MBq/mg) and [pyridine-3-^{14}C]picoxystrobin (radiochemical purity 96%; specific activity 1.9 MBq/mg) were administered by gavage in PEG 400 vehicle (4 ml/kg bw). Tissue ^{14}C residues were measured, using combustion and/or LSC, at 1, 24 and 120 hours after administration of the low (10 mg/kg bw) dose and 24, 48 and 120 hours after administration of the high (100 mg/kg bw) dose. The initial time points were chosen to approximate the T_{max}.

The results (Table 3) showed extensive absorption and systemic distribution at the low dose level, with a lower relative total body burden at the high dose level. Females typically had higher concentrations of radiolabel in samples compared with males at the first time point. Tissues with highest concentrations were liver, plasma, pancreas and kidney.

Table 3. Tissue levels of ^{14}C in rats administered [^{14}C]picoxystrobin

	Mean tissue levels (µg Eq/g)			
	10 mg/kg bw		100 mg/kg bw	
	1 h	24 h	24 h	120 h
Males				
Carcass	1.2	0.3	3.0	0.3
Plasma	2.6	0.9	7.9	0.8
Red blood cells	0.8	0.4	3.9	1.0
Fat	0.7	0.4	4.7	0.3
Liver	12	3.5	26	4.1
Pancreas	3.8	0.3	2.9	0.4
Kidney	2.8	0.9	8.6	1.3
Testes	0.3	0.1	1.4	0.2
Females				
Carcass	2.0	0.2	3.2	0.4
Plasma	6.2	0.8	9.1	0.9
Red blood cells	1.7	0.3	4.1	1.4
Fat	2.2	0.4	8.9	0.8
Liver	20	2.4	32	2.7
Pancreas	2.2	0.2	2.9	0.3
Kidney	5.6	0.7	9.3	1.7

From Himmelstein (2010)

(b) Dermal route

The ability of picoxystrobin to penetrate the skin was evaluated using a 250 g/l soluble concentrate formulation. In an in vivo rat study, less than 1% of the dose applied as the concentrated product was absorbed in 24 hours, with less than 1% found at the application site. With a 1:200 aqueous dilution, 5–21% was absorbed in 24 hours. A comparison of the absorption through rat and human skin samples in vitro showed that absorption was approximately 15- to 25-fold greater through rat skin than through human skin for both the concentrated product and the dilution. The absorption of picoxystrobin through intact human skin in vivo is likely to be low (B.K. Jones, 1999; Ward, 1999).

1.2 Biotransformation

Rats

The biotransformation of picoxystrobin was investigated in Alpk:AP$_f$SD rats receiving a single oral dose of (^{14}C-pyridinyl)-labelled picoxystrobin (radiochemical purity 97%; specific activity 1.9 GBq/mmol) or (^{14}C-phenyl)-labelled picoxystrobin (radiochemical purity 97%; specific activity 1.9 GBq/mmol) at 100 mg/kg bw in PEG 600. Metabolites were characterized by physicochemical, chromatographic and biochemical methods. Urine, bile and extracts of urine, bile and faeces were analysed by liquid chromatography with mass spectrometry and/or liquid chromatography with tandem mass spectrometry. Enzymatic digestion with β-glucuronidase was used to investigate glucuronide conjugation. When available, metabolite standards were used to confirm structural assignments. Many metabolites were isolated to purity to allow mass spectrometric, tandem mass spectrometric and nuclear magnetic resonance spectroscopic analysis. Isolated metabolites and extracts of urine and bile were subjected to methylation to confirm the presence of carboxylic acid functions. Additionally, the metabolites present in samples of excreta from low-, high- and repeated-dose mass balance/excretion studies (see Brown, 1998a,b,c above) were characterized and quantified

using mass spectrometry and/or co-chromatography with reference standards, radio–high-performance liquid chromatography or nuclear magnetic resonance.

Picoxystrobin is extensively metabolized, and 42 metabolites have been found, of which 34 have been structurally identified. All metabolites that represented greater than 5% of the administered dose were identified, with the exception of one faecal metabolite (A), which is considered to originate from bile, in which all significant metabolites were characterized. The majority of the dose (73–83%) is accounted for by identified metabolites in samples collected following single- or repeated-dose mass balance studies. Biotransformation reactions for picoxystrobin include ester hydrolysis, oxidation, *O*-demethylation, cleavage to separate the two ring structures and glucuronide conjugation. The major route of metabolism is ester hydrolysis and glucuronide conjugation and is consistent following both single and repeated daily oral doses at 10 mg/kg bw and following a single oral dose at 100 mg/kg bw. This major route of metabolism was similar in males and females, although there were some minor sex differences in metabolism. Major urinary and bile metabolites are presented in Table 4, and a metabolic pathway is shown in Figure 2.

Table 4. Main metabolites (> 2%) in urine and bile from cannulated rats administered (^{14}C-phenyl)- or (^{14}C-pyridinyl)-labelled picoxystrobin at 100 mg/kg bw[a]

Metabolite	% of administered radioactivity					
	^{14}C-phenyl		^{14}C-pyridinyl			
	Males Urine	Females Urine	Males Urine	Males Bile	Females Urine	Females Bile
02	—		—	1.02	—	2.12
02/48	—	5.75	—	—	1.72	—
40	—	3.05	—	28.87	2.23	30.14
43	—	Trace	—	6.13	—	6.04
46	—	—	—	—	—	2.14
46/49/49A	—	1.64	—	22.18	1.75	17.96
47	—	Trace	—	3.15	—	1.17
50/54	—	—	—	3.35	—	1.18
52	—	3.13	—	—	3.08	—
59	—	—	—	1.52	—	3.33
Subtotal: characterized	1.68	22.34	4.27	69.46	15.80	65.68
Subtotal: unknown	0.3	0.92	0.27	2.1	0.7	0
Total	1.98	23.26	4.54	71.56	16.50	65.68

From Macpherson (1999)

[a] (^{14}C-pyridinyl)-labelled picoxystrobin in faeces: 15.56% of administered radioactivity for males; 18.81% of administered radioactivity for females.

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

In the rat, picoxystrobin is of low acute toxicity by the oral and dermal routes, but of high toxicity by inhalation (Table 5).

Figure 2. Proposed metabolic pathway for picoxystrobin in the rat

Table 5. Acute toxicity studies with picoxystrobin

Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	LC_{50} (mg/l)	Purity; batch	Vehicle	Reference
Mouse	Crl:CD1	F	Oral	> 5000	—	99.3%; SEP07AS013	0.5% methylcellulose	Kesavan (2010)
Rat	AlPk	M/F	Oral	> 5000	—	93.3%; P25	Corn oil	Lees (1997a)
Rat	Crl:CD (SD)	F	Oral	> 5000	—	99.66%; O21	Water	Carpenter (2007)
Rat	Crl:CD (SD)	F	Oral	> 5000	—	98.7–99.9%; various	0.5% methylcellulose	Bentley (2010)
Rat	Crl:WI (Wistar)	F	Oral	> 2000	—	99.6%; JAN10AS046	Corn oil	Ramu (2010)
Rat	AlPk	M/F	Dermal	> 2000	—	93.3%; P25	Water	Lees (1997b)
Rat	AlPk	M/F	Inhalation (4 h, nose only)	—	M: 2.1–4.6 F: 3.2 (MMAD 6 µm)	93%; P10	None (aerosol)	Kilgour (1998)
Rat	Wistar	M/F	Inhalation (4 h, nose only)	—	0.11 (MMAD 3.4–4.1 µm)	SEP07AS013	None (aerosol)	Rajsekhar (2009)

F, female; LC_{50}, median lethal concentration; LD_{50}, median lethal dose; M, male; MMAD, mass median aerodynamic diameter

(b) Dermal and ocular irritation and dermal sensitization

In the rabbit, picoxystrobin is slightly irritating to skin and moderately irritating to the eye (Lees, 1997c,d). Picoxystrobin was negative in a Magnusson and Kligman maximization assay for skin sensitization in guinea-pigs (Lees, 1997e).

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

Groups of 10 male and 10 female C57BL/10J$_f$AP/Alpk mice were fed diets containing 0 (control), 200, 800, 1600 or 2400 ppm picoxystrobin (purity 99%; batch P13) for 90 consecutive days. Achieved intakes were 0, 33, 137, 291 and 422 mg/kg bw per day for males and 0, 44, 176, 359 and 535 mg/kg bw per day for females, respectively. Clinical observations, body weights and feed consumption were measured routinely. At the end of the scheduled period, the animals were sacrificed and subjected to a full postmortem examination. Selected organs were weighed, and a range of tissues was taken for subsequent histopathological examination.

Analysis of the diets showed that the achieved concentrations, homogeneity and stability were satisfactory throughout the study.

There were no deaths or clinical changes considered to be related to treatment with picoxystrobin. Over the first 4 days of the study, both sexes receiving 2400 ppm and males receiving 1600 ppm lost body weight. There were reductions in feed consumption and body weight (approximately 10%) over the entire study and increases in liver weight, after adjustment for terminal body weight, in animals at dietary concentrations of 800, 1600 or 2400 ppm picoxystrobin (Table 6). Food utilization was less efficient in animals receiving 1600 or 2400 ppm picoxystrobin. There was a treatment-related increase in the incidence of minimal hepatocyte hypertrophy in males given 1600 ppm and above and females given 800 ppm and above (Table 6). The liver and body weight findings at 800 ppm are considered to be not adverse.

Table 6. Findings in mice receiving picoxystrobin for 90 days

	0 ppm	200 ppm	800 ppm	1600 ppm	2400 ppm
Males					
Body weight (g)	29	30	29	27*	27*
Liver weight (g)	1.3	1.4	1.4	1.3	1.3
Relative liver weight (%)	4.4	4.5	4.8*	4.9*	4.9*
Hepatocyte hypertrophy	0/10	0/10	2/10	6*/10	4/10
Females					
Body weight (g)	26	25	24	24*	23*
Liver weight (g)	1.2	1.2	1.2	1.2	1.2
Relative liver weight (%)	4.8	4.7	4.9*	5.1*	5.1*
Hepatocyte hypertrophy	0/10	2/10	5*/10	6*/10	9*/10

From Rattray (1996)
* $P < 0.05$

The no-observed-adverse-effect level (NOAEL) was 800 ppm (equal to 137 mg/kg bw per day), based on reduced body weight gain and increased relative liver weight at 1600 ppm (equal to 291 mg/kg bw per day) (Rattray, 1996).

Rats

Groups of 12 male and 12 female Alpk:AP_fSD rats were fed diets containing 0 (control), 100, 500 or 1250 ppm picoxystrobin (purity 93.3%; batch P25) for 90 consecutive days. Achieved intakes were 0, 8.5, 42 and 105 mg/kg bw per day for males and 0, 10, 48 and 120 mg/kg bw per day for females, respectively. Clinical observations, body weights and feed consumption were measured, and the animals were killed at the end of the scheduled period and subjected to a full postmortem examination. Cardiac blood samples were taken and urine samples were collected for clinical pathology, selected organs were weighed and a wide range of tissues was taken from control and top-dose animals for subsequent histopathological examination.

Analysis of the diets showed that the achieved concentrations, homogeneity and stability were satisfactory throughout the study.

One male given 1250 ppm was sacrificed for humane reasons, unrelated to treatment, in week 7 of the study. All other animals survived to scheduled termination. There were no treatment-related clinical signs in any animals. Top-dose animals showed an initial decrease in body weight gain compared with concurrent controls during the 1st week of the study. At the end of the study, body weights in top-dose animals were 10% and 8% below those of concurrent male and female controls, respectively. Body weights in all other treated groups were not different from control values throughout the study (Table 7). Feed consumption was decreased (approximately 10%) throughout the study in top-dose animals compared with concurrent controls. Feed consumption in other dose groups was essentially similar to control values. Feed utilization was less efficient in top-dose females during the first 4 weeks of the study, but thereafter was similar to control values.

Table 7. Body and liver weights of rats exposed to picoxystrobin via the diet

	Mean weight (g)							
	Males				Females			
	0 ppm	100 ppm	500 ppm	1250 ppm	0 ppm	100 ppm	500 ppm	1250 ppm
Body weight								
- week 0	154.4	153.9	154.7	153.7	131.7	132.9	134.4	134.1
- week 1	206.4	208.3	204.7	192.0*	158.8	159.0	158.9	151.6*
- week 13	495.5	484.7	478.2	447.8*	274.2	277.6	268.5	251.8*
Liver weight	20.8	21.0	21.6	20.8	10.5	11.1	11.0	10.8
Liver weight adjusted for terminal body weight	19.8	20.6	21.5*	22.6*	10.3	10.7	10.9	11.5*

From Rattray (1998)
* $P < 0.05$

There were no consistent or notable changes in haematology, clinical chemistry or urine analysis findings, nor were there any treatment-related gross pathological or histopathological changes in any organs.

There was no significant increase in absolute liver weights, but an increase in relative liver weight was seen in both sexes at the top dose level and in males at 500 ppm (Table 7). In the absence of any histopathological changes in the liver, the finding at 500 ppm (< 10% change) was considered to be of no toxicological significance.

The NOAEL was 500 ppm (equal to 42 mg/kg bw per day), based on reduced body weight gain and feed consumption and increased relative liver weight at 1250 ppm (equal to 105 mg/kg bw per day) (Rattray, 1998).

Dogs

Groups of four male and four female Beagle dogs were fed diets containing 0 (control), 125, 250 or 500 ppm picoxystrobin (purity 94.4%; batch P27) for a period of 90 days. Males received 350 g diet per day and females 300 g/day. Dose rates were 0, 4.3, 8.9 and 17 mg/kg bw per day for males and 0, 4.3, 8.5 and 17 mg/kg bw per day for females, respectively. Clinical observations and veterinary examinations (including ophthalmoscopy) were made, and body weights, feed consumption and clinical pathology parameters were measured. Samples for haematology and clinical chemistry were taken pretest and at weeks 4, 8 and 13. At the end of the scheduled period, the animals were killed and subjected to a full gross pathological examination. Selected organs were weighed, and a range of tissues was taken for subsequent histopathological examination.

Analysis of the diets showed that the achieved concentrations, homogeneity and stability of picoxystrobin were satisfactory throughout the study.

There were no deaths. Slightly increased incidences of fluid faeces in males and salivation in females were seen in animals given 500 ppm, but no other clinical signs or ophthalmoscopy findings were observed. Treatment-related effects on body weight were seen for males and females given 500 ppm (Table 8). Throughout most weeks of the study, but particularly at the start, group mean feed consumption for males and females receiving 500 ppm was reduced (10–30%) in comparison with that of concurrent controls (Table 9). Slight reductions in feed consumption and transient effects on body weight at 250 ppm were not considered adverse.

Table 8. Body weights in dogs receiving picoxystrobin in the diet

Week	Mean body weight (kg)							
	Males				Females			
	0 ppm	125 ppm	250 ppm	500 ppm	0 ppm	125 ppm	250 ppm	500 ppm
1 (start)	9.23	9.18	9.10	9.20	7.95	8.05	7.95	7.83
2	9.32	9.30	9.23	8.95**	8.07	8.00	7.99	7.86*
8	10.42	10.30	10.03	9.90*	8.87	8.70	8.79	8.31**
14	11.14	11.05	10.66	10.42	9.42	9.30	9.34	8.82*

From Horner (1998)
* $P < 0.05$; ** $P < 0.01$

Table 9. Feed consumptions in dogs receiving picoxystrobin in the diet

Week	Mean feed consumption (g/dog)							
	Males				Females			
	0 ppm	125 ppm	250 ppm	500 ppm	0 ppm	125 ppm	250 ppm	500 ppm
1	350	339	318*	256**	300	295	264	221**
2	350	350	348	267**	300	300	294	251**
5	350	350	350	340	300	300	300	281
8	350	350	350	332*	300	300	300	284*
12	350	350	350	332	300	300	300	295
13	350	350	350	331	300	300	300	294

From Horner (1998)
* $P < 0.05$; ** $P < 0.01$

There were no consistent or notable changes in haematology values. Minor decreases in group mean plasma albumin and total protein were seen in top-dose animals, which are probably secondary to feed consumption and body weight deficits. A slight increase in kidney weight relative to body weight (approximately 10%) was seen in males dosed at 500 ppm, but was without any histopathological correlate.

The NOAEL was 250 ppm (equal to 8.5 mg/kg bw per day), based on body weight deficits at 500 ppm (equal to 17 mg/kg bw per day) (Horner, 1998).

Groups of four male and four female Beagle dogs were fed diets containing 0 (control), 50, 150 or 500 ppm picoxystrobin (purity 94.4%; batch P27) for a period of 1 year. Males received 350 g of feed per day and females 300 g/day. Dose rates were 0, 1.6, 4.8 and 16 mg/kg bw per day for males and 0, 1.6, 4.6 and 16 mg/kg bw per day for females, respectively. Clinical observations and veterinary examinations (including ophthalmoscopy) were made, and body weights, feed consumption and clinical pathology parameters were measured periodically before and throughout the study. At the end of the scheduled period, the animals were killed and subjected to a full postmortem examination. Selected organs were weighed, and a range of tissues was taken for subsequent histopathological examination.

Analysis of the diets showed that the concentration, stability and homogeneity of picoxystrobin in the test diets were satisfactory.

None of the animals died before the scheduled termination. Administration of 500 ppm to female dogs resulted in an increased incidence of the observation of “thin appearance”, which is related to effects on body weight at this dose level (Table 10). There was an increased incidence of “reddened gums” and of fluid faeces in males receiving 500 ppm.

Table 10. Mean body weights and feed consumption in dogs receiving picoxystrobin in the diet

	Males				Females			
	0 ppm	50 ppm	150 ppm	500 ppm	0 ppm	50 ppm	150 ppm	500 ppm
Body weight (kg)								
- week 1 (start)	9.15	9.25	8.98	8.95	7.75	7.88	7.85	7.88
- week 2	9.20	9.30	9.20	8.60*	7.80	8.08	7.93	7.65*
- week 26	10.98	11.25	10.95	9.58*	9.33	9.88	9.93	8.28*
- week 52	11.25	11.78	11.53	10.48	9.73	10.38	10.53	9.10
Feed consumption (g)								
- week 1	340	350	349	226*	296	300	288	188*

From Lees (1999a)
* $P < 0.05$

There were no clinical or ophthalmoscopic findings that were considered to be related to the administration of picoxystrobin. Dietary administration of 500 ppm to male and female dogs resulted in lower body weight, with a maximal effect in males of 11% at week 26 and in females of 15% at week 36. There were no effects on body weight at 50 or 150 ppm picoxystrobin. Reduced feed consumption was seen in both sexes at 500 ppm (Table 10). There were no treatment-related effects on haematology or clinical chemistry, nor were there any significant findings at gross pathological or histopathological examination.

Absolute and relative to body weight values for thyroid weights were higher than those of concurrent controls for females receiving 500 ppm (Table 11). There were no histopathological

changes in the thyroid, and the values at 500 ppm were reported to be well within the historical control range (0.654–0.938 g); thus, the increase in thyroid weight is considered to be of no toxicological significance.

Table 11. Thyroid weight in female dogs receiving picoxystrobin in the diet

	Mean thyroid weight (g)			
	0 ppm	50 ppm	150 ppm	500 ppm
Absolute weight	0.60	0.60	0.64	0.69
Weight adjusted for terminal body weight	0.61	0.57	0.60	0.74*

From Lees (1999a)
* $P < 0.05$

The NOAEL was 150 ppm (equal to 4.6 mg/kg bw per day), based on reduced body weight and reduced feed consumption at 500 ppm (equal to 16 mg/kg bw per day) (Lees, 1999a).

(b) Dermal application

Groups of five male and five female Alpk:AP$_f$SD rats were administered picoxystrobin (purity 93.3%; batch P25) at 0, 200, 500 or 1000 mg/kg bw per day by dermal application 5 days/week over a 28-day period (20 applications). The test material was moistened with water and applied to clipped skin, which was then covered with an occlusive dressing for 6 hours. Clinical observations, body weights and feed consumption were recorded throughout the study. At the end of the scheduled period, the animals were killed and subjected to a postmortem examination. Cardiac blood samples were taken for clinical pathology (haematology and blood clinical chemistry), selected organs were weighed and tissues were taken for histopathological examination.

Other than an apparent increase in platelets that was linked to a low control value, there were no adverse systemic effects. An increase in sloughing at the application site was noted in top-dose males.

The NOAEL was 1000 mg/kg bw per day (equal to 700 mg/kg bw per day, corrected for the non-continuous dosing), the highest dose tested (Lees, 1999b).

Groups of Crl:CD(SD) rats (10 of each sex) were exposed to picoxystrobin (purity 99.3%; batch SEP07AS013) for 6 hours/day for 28 days. Dose levels were 0, 100, 300 and 1000 mg/kg bw per day. Test material was moistened with water and applied to the shaved skin, which was then covered with a semi-occlusive wrapping. An acceptable range of investigations was performed.

There were four deaths across dose groups, attributed to the wrapping being too tight. No adverse systemic or local effects were observed.

The NOAEL was 1000 mg/kg bw per day, the highest dose tested (Carpenter, 2009).

(c) Exposure by inhalation

Groups of Crl:CD(SD) rats (five of each sex) were exposed to picoxystrobin (purity 99.3%; batch SEP07AS013) for 6 hours/day, 5 days/week, for 4 weeks. Dose levels were 0, 0.001, 0.01 and 0.025 mg/l. The mass median aerodynamic diameters were typically less than 3 µm. An acceptable range of investigations was performed.

No adverse systemic or local effects were observed. The no-observed-adverse-effect concentration (NOAEC) was 0.025 mg/l, the highest concentration tested (Rajsekhar, 2011).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Groups of 50 male and 50 female C57BL/10J$_{f}$AP Alpk mice were fed diets containing 0, 50, 200 or 800 ppm picoxystrobin (purity 93.3%; batch P25) for 80 weeks. Achieved mean intakes were 0, 6.6, 26 and 109 mg/kg bw per day for males and 0, 8.8, 36 and 145 mg/kg bw per day for females, respectively. Clinical observations, body weights and feed consumption were measured throughout the study. At week 53, blood smears were taken for haematology, but were not examined. All animals, including any found dead or killed prematurely, were subjected to a full macroscopic examination. Cardiac blood samples were taken for haematology, selected organs were weighed and a wide range of tissues from the control and top-dose groups was examined by light microscopy. In the low- and intermediate-dose groups, histopathological examinations were performed on preputial gland, salivary gland and sciatic nerves in males and on the ovaries, uterus, stomach and spleen of females.

Homogeneity, stability and content of the diets were acceptable.

Survival was similar in all groups (> 80%), and there were no clinical signs of toxicity. Reduced body weight gains (≤ 10%) were seen in top-dose males for the majority of the study (Table 12) and in top-dose females during the first half of the study. Feed consumption was similar across test and control groups. A slight (approximately 2%), but statistically significant, reduction in mean cell haemoglobin was noted in males receiving 800 ppm picoxystrobin (Table 12). Liver weight was increased by approximately 10% in top-dose males, but was without any associated pathological change. There were no notable findings on histopathological examination other than an increase in erosion and inflammation of the non-glandular stomach in females receiving 800 ppm. The finding of erosion and inflammation of the non-glandular stomach is consistent with a local effect due to the transient irritation seen in irritation studies with picoxystrobin. Total and specific tumour incidences were similar in control and treated animals.

Table 12. Findings in Alpk mice exposed to picoxystrobin for 80 weeks

	0 ppm	50 ppm	200 ppm	800 ppm
Males				
Terminal body weight (mean; g)	35.8	35.6	36.4	34.1*
Liver weight (mean; g)	4.5	4.5	4.5	4.9*
Mean cell haemoglobin (mean; pg)	15.8	15.7	15.9	15.5*
Erosion of non-glandular stomach ($n = 50$)	3	0	0	2
Inflammation of non-glandular stomach ($n = 50$)	3	0	0	5
Tumour-bearing animals ($n = 50$)	12	14	15	16
Females				
Terminal body weight (mean; g)	28.8	29.8*	28.9	28.7
Liver weight (mean; g)	1.3	1.3	1.3	1.3
Erosion of non-glandular stomach ($n = 50$)	3	1	2	7
Inflammation of non-glandular stomach ($n = 50$)	4	2	4	8
Tumour-bearing animals ($n = 50$)	16	16	22	11

From Rattray (1999a)
* $P < 0.05$

The findings seen at 800 ppm are of small magnitude and are not considered to be adverse. The NOAELs for general toxicity and carcinogenicity are both 800 ppm (equal to 109 mg/kg bw per day), the highest dose tested (Rattray, 1999a).

In a second mouse carcinogenicity study, five groups of young adult male and female Crlj:CD-1(ICR) mice (60 of each sex per group) were administered diets that contained 0 (control), 100, 600, 2400 or 4800 ppm picoxystrobin (purity 99.3%; batch SEP07AS013) for approximately 18 months. Mean achieved intakes were 0, 12, 71, 293 and 585 mg/kg bw per day for males and 0, 16, 99, 412 and 799 mg/kg bw per day for females, respectively. Body weights and feed consumption were evaluated weekly for the first 13 weeks, then every other week thereafter. Detailed clinical observations were evaluated weekly. Ophthalmological assessments were performed prior to the start of dietary exposure and near the end of the exposure period. White blood cell differential counts were evaluated in surviving mice at the end of the exposure period and in mice that were sacrificed in extremis. After approximately 18 months of dietary exposure, mice were sacrificed and given a gross and microscopic pathological examination. A full range of tissues from control and top-dose animals was investigated histopathologically, but only gross lesions, liver, duodenum and stomach were investigated from other groups.

Homogeneity, stability and content of the test diet were confirmed analytically.

No notable changes were observed in clinical observations, body weight, feed intake, ophthalmology, white blood cell differential counts or cause of death in mice exposed to picoxystrobin. Survival was significantly higher in top-dose males than in controls (Table 13). A statistically significant change in the ratio of lymphocytes to neutrophils was seen in top-dose females, but the results were within the normal large variation for this parameter. Histopathological changes of the duodenum consisted of increased incidences and severity of mucosal hyperplasia in males fed dietary concentrations of 4800 ppm, but not in females (Table 13). Increases in liver weights were observed in males and females fed dietary concentrations of 2400 ppm and above (Table 13). In female mice, the increased liver weights correlated with the test article–related microscopic finding of centrilobular hepatocellular hypertrophy (Table 13). Male mice exposed to 600 ppm picoxystrobin and higher had reduced incidences of malignant lymphoma. The incidence of liver nodules and hepatocellular adenoma was higher in the 4800 ppm male group (22%) compared with the concurrent control group (6%), which also resulted in an overall increase in the incidence of combined hepatic adenoma/carcinoma (animals with at least one hepatocellular neoplasm) (Table 13). The higher incidence of hepatocellular neoplasms was considered to be related to the marked increase in survival in the 2400 and 4800 ppm male groups (83% and 93%, respectively) compared with the control group (72%). The increase in liver tumours was statistically significant by the Cochran-Armitage trend test and Fisher's exact test (one tail), but not when adjusted for survival by either the Poly-3 test or Peto analysis. There was no increase in hepatocellular carcinoma and no increase in liver tumours of any type in females (Table 13). The incidence in hepatocellular adenoma in the high-dose males was similar to incidences (1–20%) reported by the animal supplier for a relevant time period (Charles River, 2003–2006), where the average survival at study termination was lower (77%) than that observed in this study (Giknis & Clifford, 2010). The test facility had performed three similar studies that provided historical control values (adenoma 3–15% and carcinoma 5–12%) typical of those for Charles River sourced CD-1 mice (Bentley, 2012). The overall weight of evidence suggests that the liver tumours in male mice are part of the general picture of tumours seen in aged mice.

The NOAEL for non-neoplastic effects was 600 ppm (equal to 71 mg/kg bw per day), based on increased liver weights (> 10%) at 2400 ppm (equal to 293 mg/kg bw per day). The NOAEL for carcinogenicity was 4800 ppm (equal to 585 mg/kg bw per day), the highest dose tested, as the increased incidence of liver tumours in males at 2400 and 4800 ppm is considered to be secondary to increased survival in these groups (Moon, 2011).

Table 13. Findings in CD-1 mice fed diets containing picoxystrobin for 18 months

	0 ppm	100 ppm	600 ppm	2400 ppm	4800 ppm
Males (n = 60)					
Survival to termination (%)	72	68	78	83*	93*
Body weight, week 53 (mean; g)	54	52	55	52	52
Terminal body weight (mean; g)	55	52	55	53	53
Feed intake (mean; g/mouse per day; weeks 1–13)	5.1	5.1	5.1	5.0	5.0
Absolute liver weight (mean; g)	2.9	2.9	3.1	3.3*	3.5*
Liver nodules	3	2	9	5	10*
Hepatocellular hypertrophy	2	2	1	2	2
Hepatocellular foci of alteration	1	0	3	1	7*
Hepatocellular adenoma (total / terminal kill)	6 / 6	5 / 3	9 / 8	9 / 7	13* / 13
Hepatocellular carcinoma (total / terminal kill)	6 / 5	6 / 5	6 / 4	12 / 10	9 / 9
Hepatocellular tumours (all types)	12	11	15	20*	19*
Duodenum, dilated mucosal gland	2	2	0	5	7*
Duodenum, mucosal hyperplasia	3	3	1	6	10
Females (n = 60)					
Survival to termination (%)	78	85	80	72	75
Body weight, week 53 (mean; g)	43	41	41	42	40
Terminal body weight (mean; g)	45	42	42	42	42
Feed intake (mean; g/mouse per day; weeks 1–13)	5.2	5.1	5.1	5.2	5.1
Lymphocytes (mean; % of total white blood cells)	53	ND	ND	ND	61*
Neutrophils (mean; % of total white blood cells)	44	ND	ND	ND	37*
Absolute liver weight (mean; g)	2.1	2.0	2.2	2.6	2.7*
Liver nodules	0	1	2	2	2
Hepatocellular hypertrophy	2	0	3	5	28*
Hepatocellular adenoma	0	0	1	1	0
Duodenum, dilated mucosal gland	2	0	2	3	5
Duodenum, mucosal hyperplasia	5	0	2	5	7

From Moon (2011)
ND, not determined; * $P < 0.05$

Rats

Groups of 64 male and 64 female Alpk:AP$_f$SD rats were fed diets containing 0, 50, 200 or 750 ppm picoxystrobin (purity 93.3%; batch 25) for 2 years. Achieved intakes were 0, 3.1, 12 and 46 mg/kg bw per day for males and 0, 3.8, 15 and 58 mg/kg bw per day for females, respectively. Twelve males and 12 females from each group were designated for an interim kill at 53 weeks. The remaining animals continued to termination at 105 weeks. During the study, clinical and ophthalmoscopic observations were made, and body weights and feed consumption were measured. Blood and urine samples were taken at weeks 14 and 27, interim kill, weeks 53 and 79 and termination. Throughout the study, any animals found dead or killed prematurely were subjected to a full gross postmortem examination, and tissues were taken for subsequent histopathological examination. At scheduled termination, all animals were subjected to a full gross postmortem examination, cardiac blood samples were taken, selected organs were weighed and a wide range of tissues from control and top-dose rats plus selected tissues and gross lesions from other rats were evaluated histopathologically.

Homogeneity, stability and content of the diets were confirmed analytically.

Survival was less than 50% in all male groups, but was greater in top-dose males than in controls (Table 14). There were no adverse effects of picoxystrobin on observed clinical signs, ophthalmoscopy, haematology or urine analysis. Reductions in feed consumption (approximately 5%) and body weight gain were seen at 750 ppm during the first half of the study (Table 14). Reductions were seen in serum alanine aminotransferase activities in all male groups at termination and in alkaline phosphatase activities throughout the study in the 750 ppm groups; with no associated histopathological findings, these reductions in marker enzyme activities are not considered as adverse. Liver weight adjusted for body weight was increased by 7% in top-dose males at the interim kill. Kidney weights were reduced in both top-dose groups at termination (Table 14). An unusual finding of ectopic parathyroid in the thymus was noted in top-dose males, together with slight increases in Leydig cell lesions (Table 14). The incidence of large granular lymphocyte leukaemia was increased significantly in top-dose males, the finding being seen in animals dying towards the end of the study as well as at termination, indicating that the finding was not entirely dependent on survival (Table 14; Peto analysis $P = 0.042$). The haematological results provided no indication of any alterations in leukocyte numbers, there were no related changes in other organs, such as the spleen, and a parallel finding was not present in females. Although the incidence of 7 out of 52 is outside the test facility historical control incidence for males, cited as 0–10%, it was not reproduced in a second study that utilized higher dose levels, and the weight of evidence suggests that the leukaemias are incidental findings.

The NOAEL for toxicity was 200 ppm (equal to 12 mg/kg bw per day), based on reductions in body weight gain and kidney weights at 750 ppm (equal to 46 mg/kg bw per day). The NOAEL for carcinogenicity was 750 ppm (equal to 46 mg/kg bw per day), the highest dose tested. The increase in large granular lymphocyte leukaemia in top-dose males is not considered to be an adverse effect of picoxystrobin administration (Rattray, 1999b).

In a second 2-year chronic toxicity and carcinogenicity feeding study, picoxystrobin (purity 99.3%; batch SEP07AS013) was administered to five groups of male and female Crl:CD(SD) rats (approximately 80 rats of each sex per concentration). Concentrations in the diets were 0, 50, 200, 1000 and 3500 ppm. Mean achieved intakes were 0, 2.2, 8.8, 45 and 162 mg/kg bw per day for males and 0, 2.8, 11, 57 and 203 mg/kg bw per day for females, respectively. Ten rats per group were sacrificed after approximately 1 year on study. Parameters evaluated included body weight, body weight gain, feed consumption, feed efficiency, clinical signs, clinical pathology, ophthalmology, organ weights and gross and microscopic pathology. Samples for haematology, clinical chemistry and urine analysis were taken at 3, 6 and 12 months. A full range of tissues from control and top-dose animals and animals dying during the study was examined histopathologically, but only gross lesions, testes and tissue masses with regional lymph nodes were examined from other groups sacrificed at termination.

Homogeneity, stability and content of the test diet were confirmed analytically.

Survival in the 3500 ppm male and female groups and in the 1000 ppm female group was significantly greater than in controls (Table 15). There were no adverse clinical or ophthalmological observations attributed to test substance exposure. Mean body weight and body weight gain were reduced at 1 year and overall during the study in both sexes at 3500 ppm (Table 15). These body weight changes were associated with lower mean feed consumption and feed efficiency over the 1st year, which continued for the duration of the study (Table 15). Feed consumption suffered a marked reduction of approximately 25% in all groups, including controls, at week 88, but subsequently recovered. No consistent, test substance–related effects were noted on any clinical chemistry, haematology or urine analysis parameters, macroscopic findings or incidence of masses. There were no test substance–related microscopic findings following 1 year of treatment.

Table 14. Findings in Alpk rats exposed to picoxystrobin for 105 weeks

	0 ppm	50 ppm	200 ppm	750 ppm
Males				
Survival to termination (%)	23	17	27	40*
Body weight, week 53 (mean; g)	662	665	673	633*
Terminal body weight (mean; g)	546	503	539	535
ALP (mean; IU/l), week 53	240	235	237	170*
ALT (mean; IU/l), week 105	80	36*	45*	49*
Liver weight adjusted for body weight (mean; g; interim kill)	24.6	25.1	25.0	26.4
Liver weight adjusted for body weight (mean; g; terminal kill)	24.1	22.6	23.5	23.4
Kidney weight adjusted for body weight (mean; g; terminal kill)	5.8	5.7	5.6	4.8*
Leydig cell hyperplasia	1	4	4	5
Leydig cell tumour (benign)	5	6	6	9
Ectopic parathyroid	1	1	4	7*
Large granular lymphocyte leukaemia (terminal kill)	0	0	1	3
Large granular lymphocyte leukaemia (total)	2	0	3	7*
Total animals with tumours	40	42	37	37
Females				
Survival to termination (%)	39	46	52	52
Body weight, week 53 (mean; g)	357	356	351	330*
Terminal body weight (mean; g)	370	390	384	343*
ALP (mean; IU/l), week 53	123	103	116	76*
ALT (mean; IU/l), week 105	64	64	53	52
Liver weight adjusted for body weight (mean; g; interim kill)	12.7	12.0	12.1	12.9
Liver weight adjusted for body weight (mean; g; terminal kill)	14.7	15.0	15.2	15.7
Kidney weight adjusted for body weight (mean; g; terminal kill)	3.4	3.1	3.3	3.1*
Ectopic parathyroid	0	0	0	1
Large granular cell lymphocytic leukaemia (total)	5	11	4	2
Total animals with tumours	54	57	54	55

From Rattray (1999b)
ALP, alkaline phosphatase; ALT, alanine aminotransferase; IU, international units; * $P < 0.05$

At the interim kill, testicular weights were increased at 3500 ppm (Table 15), but with no associated histopathological findings. Liver weights relative to body weight were increased (10–20%) in the top-dose groups at both interim and terminal kills (Table 15). Increases in uterine hyperplasia incidence were noted relative to concurrent controls, but either were not statistically significant or showed no dose–response relationship and were within the laboratory's historical control range. A single incidence of large granular lymphocyte leukaemia was seen in a male from the 3500 ppm group. An increased incidence of thyroid gland follicular cell adenoma was seen in males from the 1000 and 3500 ppm groups, but these showed no clear dose–response relationship (Table 15) and were well within the laboratory's historical control range. At the end of the study, statistically significant increases in the incidences of interstitial cell hyperplasia and benign adenoma in the testes

were observed in male rats at 3500 ppm (Table 15). The adenoma incidence was statistically significant by Fisher's exact test (one-tailed, P = 0.03) and Cochran Armitage trend test. A Peto analysis for survival correction gave P = 0.009, indicating that the increase was not directly associated with greater survival. Although the majority of adenomas and hyperplasia occurred in terminal or near-terminal animals and the per cent incidence of adenomas fell within the historical limits of the laboratory, the study report authors considered it likely that the increases in testicular interstitial cell adenoma and hyperplasia in the 3500 ppm males were related to exposure to the test substance.

Table 15. Findings in rats fed diets containing picoxystrobin for 24 months

	0 ppm	50 ppm	200 ppm	1000 ppm	3500 ppm
Males					
Survival to termination (%)	24	31	26	29	49*
Body weight, week 53 (mean; g)	742	749	757	730	666*
Terminal body weight (mean; g)	750	716	738	724	666
Feed intake (mean; g/rat per day; weeks 1–13)	27	27	27	27	25*
Absolute testes weight (mean; g; interim kill)	3.5	3.9	3.8	3.7	3.9*
Absolute testes weight (mean; g; terminal kill)	3.8	3.3	3.5	3.5	3.7
Liver weight relative to body weight (mean; %; interim kill)	3.0	3.0	3.1	3.1	3.3
Liver weight relative to body weight (mean; %; terminal kill)	2.6	2.9	2.7	2.9	3.2*
Testes, interstitial cell tumour, benign	1	1	0	2	7*
Testes, interstitial cell hyperplasia	1	2	1	1	8*
Thyroid follicular cell adenoma	0	0	0	3	2
Females					
Survival to termination (%)	20	14	21	30*	51*
Body weight, week 53 (mean; g)	427	447	428	438	358*
Terminal body weight (mean; g)	490	493	528	465	406*
Feed intake (mean; g/rat per day; weeks 1–13)	20	20	20	20	18*
Liver weight relative to body weight (mean; %; interim kill)	3.2	3.1	3.1	3.1	3.6*
Liver weight relative to body weight (mean; %; terminal kill)	2.9	3.1	2.9	3.2	3.6*

From Craig (2011)
* $P < 0.05$

The NOAEL was 1000 ppm (equal to 45 mg/kg bw per day), based on testicular interstitial cell hyperplasia and benign adenoma, reduced body weights and increases in relative liver weights at 3500 ppm (equal to 162 mg/kg bw per day). The NOAEL for carcinogenicity was 3500 ppm (equal to 162 mg/kg bw per day), as the testicular tumours were benign adenomas (Craig, 2011).

No studies to investigate the mechanism of the testicular tumours have been provided, but a generic proposal relating to a postulated mechanism for testicular tumour production was submitted. The fungicidal activity of picoxystrobin and other strobilurin fungicides (to block mitochondrial electron transport at the Q_o site of complex III, reducing ATP production and inhibiting cellular respiration) is the same biochemical mechanism that has been demonstrated to inhibit luteinizing

hormone (LH)–induced testosterone production in the rat and in human Leydig cells in vitro (Bartlett et al., 2002; Sudisha et al., 2005; Allen et al., 2006; Midzak et al., 2007). Inhibition of LH-stimulated testosterone production leads to a compensatory increase in LH via homeostatic feedback loops through the hypothalamic–pituitary–gonadal axis, and increased LH levels are known to eventually produce Leydig cell hyperplasia and adenoma in rats if sustained (Walsh, 1977; Neumann, 1991; Bar, 1992; Ewing, 1992; Prentice & Meikle, 1995). Picoxystrobin is not genotoxic (see section 2.4) and did not induce tumours of any other type in rats or in mice. The observed increase in Leydig cell hyperplasia and adenomas in the recent 2-year rat study occurred only at the high dose, where a clear threshold response was evident by a non-linear dose–response relationship at only the very highest level of 3500 ppm. The increase in Leydig cell hyperplasia and adenomas occurred in only one species (in rats and not in mice), consistent with the known response of these species to chronic elevations in LH (Cook et al., 1999). Further, the increases were not evident following 1 year of chronic daily exposure, but only following a lifetime or near-lifetime (2 years) chronic daily exposure. All animals displaying Leydig cell hyperplasia and/or adenoma were those that survived to the end of the study or died late in the study. This delayed onset is consistent with the known pathological timeline for the induction of LH-mediated Leydig cell hyperplasia and adenoma.

There are no data specific to picoxystrobin relating to this proposed mechanism. Other than effects on testes, there were neither pathological nor clinical chemistry findings to support any disruption of the hypothalamic–pituitary–gonadal axis (most test and control rats had pituitary tumours). No data on ATP, LH or testosterone levels in rats administered picoxystrobin were submitted.

2.4 Genotoxicity

Testing of the genotoxicity of picoxystrobin has been performed in a range of assays (Table 16). Positive results were seen at cytotoxic concentrations in a reverse mutation assay with mouse lymphoma L5178Y cells in the presence of metabolic activation. The remaining in vitro and the two in vivo tests were negative. The overall database is considered adequate to conclude that picoxystrobin is not genotoxic.

2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

Rats

In an initial reproductive toxicity study, groups of 26 male and 26 female weanling Alpk:AP$_f$SD rats were fed diet containing 0 (control), 50, 200 or 750 ppm picoxystrobin (purity 93.3%; batch P25). After 10 weeks, the animals were mated and allowed to rear the ensuing F_1 litters to weaning. The breeding programme was repeated with the F_1 parents selected from the F_1 offspring to produce the F_2 litters after a 10-week premating period. Test diets were fed continuously throughout the study. Routine assessments were performed of clinical signs, body weight, feed consumption, estrous cycling, mating performance and duration of gestation. A range of pup parameters was investigated, including pup weight and live pup numbers. Litters were not culled. At termination, all parental rats, 5 pups per F_1 group and 10 pups per F_2 group received a necropsy examination, and liver, testes and epididymides were weighed. Microscopic examinations were performed on a range of tissues from all pups that were necropsied and from control and top-dose parents. There were no investigations of sperm parameters or developmental landmarks.

Homogeneity, stability and content of test diets were confirmed. Achieved doses are given in Table 17.

Table 16. Results of studies of genotoxicity with picoxystrobin

Test substance (vehicle)	End-point	Test object	Concentration	Purity; batch	Result	Reference
In vitro						
Picoxystrobin (DMSO)	Reverse mutation	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537; *Escherichia coli* WP2*uvrA*	100–5000 µg/plate, with and without S9 mix	93.3%; P-25	Negative	Callander (1996)
Picoxystrobin (DMSO)	Reverse mutation	Mouse lymphoma L5178Y cells	4–75[a] µg/ml with and without S9 mix	93.3%; P-25	Negative −S9 Weak positive +S9	Clay (1996)
Picoxystrobin (DMSO)	Chromosomal aberration	Human lymphocytes	4–60[b] µg/ml, with S9 mix 0.5–5[b] µg/ml, without S9 mix	93.3%; P-25	Negative	Fox & Wildgoose (1996)
In vivo						
Picoxystrobin (corn oil)	Bone marrow micronuclei	Alpk mice (M/F)	0, 2000, 3200 or 5000[c] mg/kg bw (gavage) single	93.3%; P-25	Negative	Fox & Mackay (1996)
Picoxystrobin (corn oil)	Liver unscheduled DNA synthesis	Alpk Rats (M)	0, 3200 or 5000 mg/kg bw (gavage) single	93.3%; P-25	Negative	Mackay (1996)

DMSO, dimethyl sulfoxide; DNA, deoxyribonucleic acid; F, female; M, male; S9, 9000 × *g* supernatant fraction of rat liver homogenate

[a] Solubility limit in treatment medium.

[b] Cytotoxicity.

[c] Marked clinical signs and altered polychromatic erythrocyte:normochromatic erythrocyte ratio.

No test substance–related mortality or clinical signs of toxicity were observed. There were no effects on mating performance, fertility, litter sizes, litter sex ratio, pup birth weights or other reproductive end-points (Table 17). Reductions in parental body weights and pup/litter weights were seen at 750 ppm; although these were statistically significant at some time points, they were small in magnitude (< 10%; Table 17). There were no treatment-related findings in the macroscopic or microscopic examinations. An increase in liver weights corrected for body weights at 750 ppm is not considered adverse, as the magnitude was less than 10% and there were no associated pathological findings.

The NOAEL for reproductive toxicity was 750 ppm (equal to 78 mg/kg bw per day), the highest dose tested. The NOAEL for parental and offspring toxicity was 200 ppm (equal to 21 mg/kg bw per day), based on reductions in body weight gain at 750 ppm (Milburn, 1998).

Table 17. Findings in a reproductive toxicity study with picoxystrobin

	0 ppm		50 ppm		200 ppm		750 ppm	
	M	F	M	F	M	F	M	F
1st mating								
Mean picoxystrobin intake (mg/kg bw per day)	0	0	5.3	5.8	21	23	78	86
Parental body weight (mean; g; day 70)	436	249	437	252	425	246	403*	234*
Maternal body weight (mean; g; LD 15)	—	351	—	357	—	349	—	332*
Dams with live pups	—	21	—	21	—	26	—	22
Mean live pups/litter (day 1)	11.0		11.2		12.1		10.1	
Mean live pups/litter (day 22)	9.8		9.3		10.9		9.0	
Pup weight (mean; g; day 1)	6.0	5.7	6.0	5.6	6.1	5.8	6.3	5.9
Pup weight (mean; g; day 22)	45	44	47	46	45	43	45	44
Litter weight (mean; g; day 22)	421		405		466		384	
Liver weight adjusted for body weight (mean; g)	20	15	21	14	22*	15	23*	16*
2nd mating								
Mean picoxystrobin intake (mg/kg bw per day)	0	0	5.4	5.8	22	24	82	89
Parental body weight (mean; g; day 70)	437	246	430	255	412*	246	404*	230*
Maternal body weight (mean; g; LD 15)	—	346	—	353	—	350	—	329
Dams with live pups	—	22	—	24	—	23	—	24
Mean live pups/litter (day 1)	10.9		11.0		11.2		12.0	
Mean live pups/litter (day 22)	10.4		10.1		10.3		11.3	
Pup weight (g; day 1)	6.7	6.3	6.3*	6.1	6.5	6.1	6.3	5.9
Pup weight (g; day 22)	45	43	45	44	44	42	38*	37*
Litter weight (g; day 22)	440		437		436		405	
Liver weight adjusted for body weight (g)	21	14	21	14	21	14	23*	15

From Milburn (1998)
F, female; LD, lactation day; M, male; * $P < 0.05$

In a second multigeneration reproductive toxicity study, groups of 30 male and 30 female Crl:CD(SD) rats were fed diet containing 0 (control), 75, 300, 1000 or 2500 ppm picoxystrobin technical (purity 99.3%; batch SEP07AS013). After approximately 70 days, the animals were mated to generate F_1 offspring. F_1 generation rats were given continued access to the same test diet concentrations as their respective P_1 generation sires and dams beginning at weaning and were subsequently mated to produce the F_2 generation. Routine assessments were performed of clinical signs, body weight, feed consumption, estrous cycling, sperm viability, mating performance and duration of gestation. An extensive range of pup parameters was investigated, including pup weight, live pup numbers and time to sexual maturation. Litters were culled to five of each sex on day 5 after birth. Necropsy examinations were performed on all parental animals, one pup of each sex per litter from the day 5 cull and pups discarded on day 22. Major organs and reproductive organs from 10 parental rats of each sex per group were weighed. Histopathological examinations were performed on a wide range of tissues from 10 rats of each sex from the control and top-dose groups. In addition, reproductive organs and thymuses from all first-mating females were examined histopathologically.

Homogeneity, stability and content of test diets were confirmed. Achieved doses are given in Table 18.

Table 18. Findings in a reproductive toxicity study with picoxystrobin

	0 ppm		75 ppm		300 ppm		1000 ppm		2500 ppm	
	M	F	M	F	M	F	M	F	M	F
1st mating										
Mean picoxystrobin intake (mg/kg bw per day)	0	0	4.0	5.4	16	22	52	70	130	173
Parental body weight (mean; g; day 70)	572	304	574	314	569	313	552	297	537*	284*
Maternal body weight (mean; g; LD 15)	—	369	—	377	—	368	—	366	—	344*
Feed consumption (mean; g/rat per day; days 1–70)	27	20	28	20	27	19	26	19	26	19
Feed consumption (mean; g/rat per day; LDs 1–15)	—	132	—	135	—	132	—	127	—	119*
Dams with live pups	—	26	—	23	—	29	—	28	—	27
Mean live pups/litter (day 1)	14.6		14.3		15.0		12.9*		14.1	
Mean live pups/litter (day 22)	7.8		8.0		8.0		8.0		7.9	
Abnormal sperm (mean; %)	6.2	—	8.3	—	4.4	—	4.8	—	5.5	—
Pup weight (mean; g; day 1)	6.8		7.0		6.8		7.0		6.8	
Pup weight (mean; g; day 15)	33.6		36.2		33.9		31.2		25.8*	
Liver weight (mean; g)	22.5	17.4	22.4	16.9	23.0	16.8	23.3	17.0	23.5	18.4*
Thymic atrophy ($n = 10$)	0	0	NE	0	NE	0	NE	0	0	6*[a]
Relative thymus weight (mean; % body weight)	0.04	0.05	0.04	0.06	0.04	0.05	0.04	0.05	0.04	0.04*
Ratio pup thymus:brain weight (mean; %)	14.5	16.8	15.0	17.5	15.6	16.6	14.9	15.4	10.5*	11.4*
Ratio pup spleen:brain weight (mean; %)	18.1	18.4	18.2	18.9	18.0	17.4	16.3	16.0*	12.8*	13.5*
2nd mating										
Mean picoxystrobin intake (mg/kg bw per day)	0	0	5.3	7.9	21	32	71	106	188	273
Parental body weight (mean; g; day 86)	498	284	505	295	500	290	479	271	446*	256*
Maternal body weight (mean; g; LD 15)	—	364	—	376	—	373	—	357	—	342*
Feed consumption (mean; g/rat per day; days 22–86)	28	21	28	22	28	21	27	20	26*	19*
Feed consumption (mean; g/rat per day; LDs 1–15)	—	48	—	48	—	51	—	47	—	45
Dams with live pups	—	27	—	28	—	24	—	28	—	27
Mean live pups/litter (day 1)	15.2		14.5		15.3		14.5		14.6	
Mean live pups/litter (day 22)	7.9		7.8		8.0		7.9		7.7	
Abnormal sperm (mean; %)	8.5	—	4.1*	—	3.9*	—	3.1*	—	3.7*	—
Pup weight (mean; g; day 1)	6.4		6.6		6.8		6.8		6.8	
Pup weight (mean; g; day 15)	34		35		36		34		30*	
Liver weight (mean; g)	26.7	17.6	27.1	17.2	26.5	18.1	26.7	16.8	27.3	18.5
Thymic atrophy ($n = 10$)	0	0	NE	NE	NE	NE	NE	NE	0	1

Table 18 (continued)

	0 ppm		75 ppm		300 ppm		1000 ppm		2500 ppm	
	M	F	M	F	M	F	M	F	M	F
Relative thymus weight (mean; % body weight)	0.06	0.07	0.06	0.07	0.06	0.06	0.06	0.06	0.06	0.06
Ratio pup thymus:brain weight (mean; %)	15.4	16.3	14.5	14.4	15.2	15.7	14.9	15.2	11.3*	11.9*
Ratio pup spleen:brain weight (mean; %)	17.1	17.1	16.9	17.1	17.6	18.2	15.7	15.4	13.6*	13.3*

From Barnett (2010)

F, female; LD, lactation day; M, male; NE, not examined; * $P < 0.05$

[a] 11/30 on further examination.

No test substance–related mortality or clinical signs of toxicity were observed. There were no effects on mating performance, fertility, litter sizes, litter sex ratio, pup birth weights or other reproductive end-points (Table 18). Slight extension of time to sexual maturation at 2500 ppm was secondary to the lower body weights in this group. Reductions in body weight, body weight gain and feed consumption were observed in the P_1 and F_1 generation 2500 ppm male and female rats (Table 18). The 1000 ppm F_1 females also had slightly reduced mean body weight and feed consumption during the 1st week of exposure, but this did not persist and is not considered adverse. Pup weights at birth were similar across all groups; subsequently, weights of pups from the 2500 ppm groups were significantly reduced on postnatal day 8 (PND 8) (F_1 pups only), PND 15 and PND 22. F_1 generation pup weights were also significantly reduced (by 8%) at 1000 ppm on PND 22.

Absolute and/or relative liver weights were increased in 1000 ppm P_1 males and 2500 ppm P_1 and F_1 males and females (Table 18), but without any associated histopathological findings. Absolute and relative thymus weights were reduced at 2500 ppm, and this was associated with an increase in thymic atrophy in first-mating dams. There were no other remarkable findings in the macroscopic or microscopic examinations.

The NOAEL for reproductive toxicity was 2500 ppm (equal to 130 mg/kg bw per day), the highest dose tested. The NOAEL for parental and offspring toxicity was 1000 ppm (equal to 52 mg/kg bw per day), based on body weight deficits and thymic atrophy at 2500 ppm (Barnett, 2010).

(b) Developmental toxicity

Rats

Groups of 24 time-mated female Alpk:AP$_f$SD rats were dosed orally by gavage with 0 (corn oil; 10 ml/kg bw), 10, 30 or 100 mg/kg bw per day (purity 99%; batch P9) on days 7–16 (inclusive) of gestation. Achieved doses were confirmed analytically. Dams were observed regularly for clinical signs, body weight and feed consumption. On day 22 of gestation, dams were killed and the uterine contents examined and removed. Fetuses were assessed for viability and external, visceral and skeletal abnormalities. The study report contained no individual animal data.

One female given picoxystrobin at a dose of 30 mg/kg bw per day was terminated on day 10 of the study for humane reasons, unrelated to treatment, following an accidental injury. The only clinical observations related to treatment with picoxystrobin were an increased incidence of diarrhoea/signs of diarrhoea and urine staining at 100 mg/kg bw per day and a slight increase in the incidence of salivation at 100 and 30 mg/kg bw per day. Administration of 100 mg/kg bw per day was associated with a reduction in body weight gain from the onset of dosing to termination (Table 19). Feed consumption was also reduced in animals given 100 mg kg bw per day throughout the dosing period (Table 19).

Table 19. Findings in rats exposed to picoxystrobin during fetal development

	0 mg/kg bw per day	10 mg/kg bw per day	30 mg/kg bw per day	100 mg/kg per bw day
Body weight (mean; g)				
- day 7	287.5	290.8	282.3	286.8
- day 8	288.7	290.4	289.2	279.2*
- day 11	306.5	307.7	305.0	297.1*
- day 19	381.3	387.0	382.1	365.3*
- day 22	396.4	392.7	407.4*	387.6
Feed consumption (mean; g/rat per day)				
- days 7–10	22	24	21	15*
- days 13–16	26	27	26	23*
- days 19–22	24	22	28	27
Mean live fetuses/litter	12.7	14.9*	13.0	14.0
3rd centrum not ossified				
- number of pups affected (%)	16 (5.2)	30 (8.4)	11 (3.8)	32* (9.6)
- number of litters affected (%)	8 (33.3)	11 (45.8)	5 (22.7)	12 (50.0)
5th sternebrae misaligned				
- number of pups affected (%)	0 (0)	0 (0)	1 (0.3)	5* (1.5)
- number of litters affected (%)	0 (0)	0 (0)	1 (4.5)	5* (20.8)

From Moxon (1998)
* $P < 0.05$

There were no adverse effects of picoxystrobin on number, weight, growth or viability of fetuses in utero. There was no treatment-related effect on the type or incidence of major fetal defects. The proportion of fetuses with minor external/visceral defects was similar for all groups, including the controls. A statistically significant difference was seen in the number of fetuses in the 100 mg/kg bw per day group with specific skeletal anomalies: 3rd cervical centrum, unossified; and 5th sternebra, misaligned (Table 19). The historical control data for the test facility show that misaligned 5th sternebrae are commonly seen in individual fetuses from litters, although the incidences seen in the picoxystrobin study are outside the historical control range for fetuses (0–1.4%) and litters (0–12.5%). Unossified 3rd centrum is a common finding and, although statistically significant at the top dose level, is considered not to be a treatment-related adverse finding. There was no evidence for a treatment-related effect on the proportion of fetuses with external/visceral variants or on the *manus* and *pes* assessment.

The NOAEL for teratogenicity was 100 mg/kg bw per day, the highest dose tested. The NOAEL for developmental toxicity was 30 mg/kg bw per day, based on an increase in misaligned 5th sternebrae at 100 mg/kg bw per day. The NOAEL for maternal toxicity was 30 mg/kg bw per day, based on reduced body weight and feed consumption during the dosing period at 100 mg/kg bw per day (Moxon, 1998).

Rabbits

Groups of 20 time-mated, female New Zealand White rabbits were dosed orally by gavage with picoxystrobin (purity 93.3%; batch P25) at doses of 0 (1% carboxymethylcellulose, 1 ml/kg bw), 8, 25 or 100 mg/kg bw per day on days 8–20 (inclusive) of gestation. Achieved doses were confirmed analytically. Dams were observed regularly for clinical signs, body weight and feed consumption. On day 30 of gestation, dams were killed and the uterine contents examined and removed. Fetuses were

assessed for viability and external, visceral and skeletal abnormalities. The study report contained no individual animal data.

All animals survived the duration of the study. Administration of 100 mg/kg bw per day was associated with reduced body weight and feed consumption during the early phases of dosing (Table 20). Incidences of "signs of diarrhoea" and "reduced faecal output" were also increased at 100 mg/kg bw per day. Transient body weight loss and reduced feed consumption were also seen at 25 mg/kg bw per day (Table 20). Macroscopic examination of the dams post mortem revealed no treatment-related findings.

Table 20. Findings in rabbits exposed to picoxystrobin during fetal development

	0 mg/kg bw per day	8 mg/kg bw per day	25 mg/kg bw per day	100 mg/kg bw per day	Historical control
Body weight (mean; kg)					
- day 8	3.73	3.68	3.78	3.82	
- day 9	3.73	3.70	3.74	3.73*	
- day 11	3.73	3.72	3.74	3.68*	
- day 20	3.86	3.88	3.90	3.90	
- day 30	4.08	4.13	4.14	4.07	
Feed consumption (mean; g/rabbit per day)					
- days 8–11	134	142	96*	49*	
- days 17–20	167	162	147	146	
- days 23–26	141	144	132	161*	
Dams with live litters	17	16	18	16	
Corpora lutea (mean/dam)	11.1	11.3	11.6	10.2	
Implantations (mean/dam)	9.8	9.6	108	8.1*	
Preimplantation loss (total/litters affected) (%)	21/10 (12)	26/11 (14)	13/8 (6)	33*/14 (21*)	
Post-implantation loss (%)	14	7*	12	15	
Mean litter weight (g)	359	374	387	305	
Live fetuses (total/litter mean)	142/8.4	144/9.0	172/9.6	111/6.9*	
Major skeletal anomalies (fetuses/litters)	6/4	2/2	1/1	0/0	
Skeletal variants (fetuses/% fetuses)	108/74	109/74	144/84	96/84*	
Odontoid incompletely ossified (% fetuses) (fetuses/litters)	0.7 (1/1)	1.4 (2/1)	3.5 (6/4)	9.0* (10*/3)	1–8%
27 pre-pelvic vertebrae, bilateral (% fetuses) (fetuses/litters)	16.9 (24/10)	20.8 (30/9)	18.6 (32/13)	39.6* (44*/15*)	15–37%
Rib 13 long length (% fetuses) (fetuses/litters)	32.4 (46/15)	36.1 (52/11)	50* (86*/18)	53.2* (59*/14)	17–55%

From Moxon (1999)
* $P < 0.05$

Picoxystrobin did not alter the weight, growth or viability of the fetuses in utero, no dams aborted, there were no dams with total litter loss and there was no evidence of teratogenicity. A reduction in the number of live fetuses and litter weight at 100 mg/kg bw per day (Table 20) appeared to be secondary to a reduction in numbers of corpora lutea and implantations, both of which occurred before the start of the dosing period; post-implantation loss was unaffected. Evaluation of the major and minor external/visceral fetal defects and variants provided no evidence of an adverse effect of

picoxystrobin on development. Evaluation of the minor skeletal defects and variants revealed three findings (Table 20). Odontoid incompletely ossified is normally a low-incidence finding in the New Zealand White rabbit. The incidence of fetuses with an incompletely ossified odontoid in the 100 mg/kg bw per day group was slightly, but statistically significantly, increased compared with concurrent controls. The findings were concentrated in three litters, but as no individual litter data are available, it is not possible to determine whether the affected fetuses came from dams exhibiting maternal toxicity. The two other skeletal findings, 27th pre-pelvic vertebrae and 13th thoracolumbar ribs of long length, are relatively common findings in the New Zealand White rabbit. The incidence of fetuses with 27th pre-pelvic vertebrae (combined to include unilateral and bilateral) was increased in the 100 mg/kg bw per day group only (fetal incidence 44.1%, litter incidence 93.8%) and was of a slightly higher incidence than seen in control rabbits in the testing laboratory (fetal incidence 14.6–36.5%, litter incidence 52.9–89.5%). The incidence of fetuses with 13th thoracolumbar ribs of long length was apparently increased in the 100 mg/kg bw per day group and also in the 25 mg/kg bw per day group. The incidence of this finding in both groups was within the range seen previously in control rabbits in the laboratory (fetal incidence 17.1–55.2%, litter incidence 50–100%). The available historical control data come from nine studies performed from 1992 to 1998; however, only one of these studies used rabbits from the same supplier as used for the picoxystrobin study. At 100 mg/kg bw per day, the incidence of 13th thoracolumbar ribs of long length, although within the range of historical control values, coincided with a slightly increased incidence of 27th pre-pelvic vertebrae; for this reason, an association with treatment at 100 mg/kg bw per day cannot entirely be dismissed. At 25 mg/kg bw per day, the statistically significant increase in incidence of 13th thoracolumbar ribs of long length was within the test facility historical control values, was not associated with any other skeletal findings at this dose level and is considered not to be an adverse effect of treatment with picoxystrobin.

The NOAEL for teratogenicity was 100 mg/kg bw per day, the highest dose tested. The NOAEL for developmental toxicity was 25 mg/kg bw per day, based on an increase in skeletal anomalies at 100 mg/kg bw per day. The NOAEL for maternal toxicity was 25 mg/kg bw per day, based on reduced body weight and feed consumption during the dosing period at 100 mg/kg bw per day (Moxon, 1999).

2.6 Special studies

(a) Neurotoxicity

Acute neurotoxicity

Crl:CD(SD) rats (12 of each sex per group) received picoxystrobin (purity 99.3%; batch SEP07AS013) in a single oral gavage dose of 0, 200, 1000 or 2000 mg/kg bw, using 0.5% methylcellulose as vehicle. Achieved doses were confirmed by analysis. Regular, routine observations included clinical signs and appearance, body weight and feed consumption. An extensive functional observational battery was performed pretest and on days 1 (2 hours), 8 and 15; motor activity was monitored over a 90-minute session on each observation day. At sacrifice on day 16, six animals of each sex were anaesthetized and perfused; brain and spinal cord were removed. Peripheral nerve samples were prepared from six rats of each sex per group.

Three females dosed with 2000 mg/kg bw died. Clinical signs of toxicity, including reduced body temperature, reduced levels of arousal, palpebral closure and abnormal posture, were seen in animals from the 1000 and 2000 mg/kg bw groups. Transient reductions in body weight, body weight gain and feed consumption were seen in all treated groups (Table 21). Decreased motor activity was recorded in the first half of the 90-minute observation period on day 1 in all test groups (Table 21), but not on day 8 or 15. There were no abnormal findings in the neuropathological examinations.

Table 21. Body weight, feed consumption and motor activity in rats receiving picoxystrobin

	0 mg/kg bw		200 mg/kg bw		1000 mg/kg bw		2000 mg/kg bw	
	M	F	M	F	M	F	M	F
Body weight (g)								
- day 1	290	189	290	188	289	181	292	187
- day 2	292	190	284	185	280	178	276	181
- day 8	340	214	340	209	326	203	327	207
- day 15	384	232	386	229	373	223	379	231
Body weight gain (g)								
- days 1–2	1.8	1.1	−6.2*	−2.6*	−9.0*	−3.1*	−15.3*	−5.8*
- days 2–8	49	25	56	24	46	25	51	27
Feed consumption (g/rat per day)								
- days 1–2	26	20	18*	13*	12*	9*	10*	8*
- days 2–8	29	22	31	21	29	21	29	20
Movement duration index (mean (SD))								
- day 1, total	1009 (317)	819 (330)	641* (234)	752 (254)	428* (147)	511 (240)	303* (146)	335* (192)
- day 1, 0–10 min	395 (49)	355 (58)	326* (83)	328 (77)	235* (87)	241* (102)	144* (59)	135* (70)
- day 1, 10–20 min	286 (85)	221 (105)	138* (66)	133* (84)	56* (76)	81* (99)	16* (25)	58* (77)
- day 1, 20–30 min	193 (64)	113 (91)	64* (84)	72 (70)	28* (56)	42* (63)	17* (27)	16* (32)

From Malley (2010)
F, female; M, male; SD, standard deviation; * $P < 0.05$

The NOAEL for neuropathy was 2000 mg/kg bw, the highest dose tested. For general toxicity, a NOAEL could not be identified, based on decreases in motor activity and body weight at 200 mg/kg bw, the lowest dose tested (Malley, 2010).

To obtain an indication of where the NOAEL for the acute neurotoxicity study might lie, the Meeting performed basic benchmark dose modelling using the United States Environmental Protection Agency's (USEPA) benchmark dose software (BMDS version 2.2). The modelled data were the mean and standard deviation for the movement duration index in males for each of the first three time periods on day 1, a sensitive, relevant end-point with a clear dose–response relationship. Individual animal values were not used, and no correction was made for pretest values (there were no notable differences in pretest values between test and control groups). A suite of continuous models was run with a benchmark response of a 5% relative variation and a 95% confidence level for the BMD_L. The model that gave the lowest values for benchmark dose (BMD) and lower-bound confidence limit on the benchmark dose (BMD_L), which could not be dismissed, was a Hill model. The obtained values (mg/kg bw) were: session 1, BMD 79.7, BMD_L 35.6; session 2, BMD 10.4, BMD_L 5.7; and session 3, BMD 4.6, BMD_L 0.8. The BMD values (particularly for session 3) that were generated involved modelling over an order of magnitude outside the limits of the dose–response data, and the true shape of the dose–response curve is unknown; therefore, the BMD and BMD_L values should not be taken as anything more than indicating that the NOAEL in the acute neurotoxicity study of Malley (2010) is much lower than 200 mg/kg bw.

Repeated-dose neurotoxicity

Crl:CD(SD) rats (12 of each sex per dose group) received picoxystrobin (purity 99.3%, batch SEP07AS013) in the diet at a concentration of 0, 100, 600 or 3500 ppm for 14 weeks. The overall mean daily intakes were 0, 6, 36 and 207 mg/kg bw per day for males and 0, 8, 46 and 246 mg/kg bw per day for females, respectively. Regular, routine observations included clinical signs and appearance, body weight and feed consumption. An extensive neurobehavioural test battery, consisting of motor activity and functional observational battery assessments, was conducted on all study rats prior to test substance administration in order to obtain baseline measurements. This neurobehavioural test battery was conducted again during weeks 4, 8 and 13. On test days 97 and 98, six rats of each sex per group were anaesthetized and subjected to whole-body in situ perfusion for neuropathology examination. A wide range of brain, spinal cord, peripheral nerve and skeletal muscle sections was prepared and stained using haematoxylin and eosin, periodic acid Schiff or luxol fast blue, singly or in combination. Sections from control and top-dose animals were examined microscopically.

Achieved doses and homogeneity were confirmed by analysis.

Body weights in animals from the 3500 ppm group were approximately 15–25% lower than those of controls at week 13. Overall feed consumption and feed efficiency in male and female rats were reduced approximately 15–20% at 3500 ppm. There were no test substance–related adverse effects on survival, clinical signs of toxicity, motor activity, temperature, behavioural parameters, or gross pathological or neuropathological examination in males or females administered any concentration of the test substance. Males administered 3500 ppm of the test substance exhibited statistically significant reductions in forelimb and hindlimb grip strength during week 8 and forelimb grip strength during week 13. These changes are probably secondary to decreases in body weight, because the reductions were small (6–9%) relative to the body weight effects, were reported to be within the typical background values and were not associated with any significant indication of neurotoxicity.

The NOAEL for neuropathy and neurotoxicity was 3500 ppm (equal to 207 mg/kg bw per day), the highest dose tested. The NOAEL for general toxicity was 600 ppm (equal to 36 mg/kg bw per day), based on body weight effects at 3500 ppm (Anand, 2010).

(b) Immunotoxicity

The potential of picoxystrobin (purity 99.3%; batch SEP07AS013) to suppress the primary humoral response to sheep red blood cells (sRBCs) was assessed in Crl:CD1 (ICR) mice and in Crl:CD (SD) rats. Groups of 10 animals of each sex were administered the test substance for 4 weeks at a dietary level of 0 (control), 100, 600, 2400 or 4800 ppm for mice and 0, 50, 200, 1000 or 3500 ppm for rats. In mice, the overall mean daily intakes in the 0, 100, 600, 2400 and 4800 ppm groups were calculated to be 0, 16, 95, 358 and 727 mg/kg bw per day for males and 0, 20, 127, 449 and 931 mg/kg bw per day for females, respectively. In rats, the overall mean daily intakes in the 0, 50, 200, 1000 and 3500 ppm groups were calculated to be 0, 3.5, 15, 68 and 231 mg/kg bw per day for males and 0, 3.9, 16, 75 and 229 mg/kg bw per day for females, respectively. Body weights, feed consumption measurements and clinical observations were recorded during the in-life period. Prior to sacrifice, the immune system was stimulated on test day 23 by injecting sRBCs into the tail vein. Blood samples were collected from each rat on test day 28, and serum samples were assayed for their concentrations of sRBC-specific immunoglobulin M (IgM) antibodies. Contemporary data from a positive control immunosuppressive agent (cyclophosphamide, 25 mg/kg bw per day) provide confirmation that the assay performance was acceptable for detection of immunosuppression. At sacrifice, each animal was examined grossly, and selected organs were weighed (brain, spleen and thymus). No histopathological examinations were performed.

Homogeneity, stability and content of the diet were confirmed by analysis.

No clinical signs of systemic toxicity were observed. There were no statistically significant or adverse effects on mean body weight, mean body weight gain or nutritional parameters in male or

female mice at any dose level or in male or female rats fed 0, 50, 200 or 1000 ppm picoxystrobin. Lower mean body weight (approximately 6–10%), body weight gain, feed consumption and feed efficiency were observed in male and female rats fed 3500 ppm (Table 22) and are considered to be adverse. No significant adverse effects were observed on 1) gross pathology; 2) absolute and relative brain, spleen and thymus weights; or 3) humoral immune response (Table 22).

Table 22. Body weight and anti-sRBC IgM titres in mice and rats treated with picoxystrobin

	M	F	M	F	M	F	M	F	M	F
Mice										
Dietary concentration (ppm)	0		100		600		2400		4800	
Body weight (g)										
- day 0	31	23	30	23	30	22	30	23	30	23
- day 7	32	25	32	25	32	24	32	25	31	24
- day 28	35	27	35	28	36	27	35	27	33	27
IgM[a]	10.7	11.0	10.1	11.2	11.0	10.4	10.7	10.9	9.7	10.4
Rats										
Dietary concentration (ppm)	0		50		200		1000		3500	
Body weight (g)										
- day 0	245	189	244	189	246	189	246	190	245	190
- day 7	296	207	301	207	304	212	293	207	269*	191*
- day 28	400	245	415	242	413	248	397	243	366*	224*
IgM[a]	13.7	14.0	14.4	15.5	14.5	15.0	13.4	14.7	14.5	14.7

From Hoban (2010a,b)
F, female; M, male; * $P < 0.05$
[a] Mean $\log_2$ of the sRBC-specific serum IgM titre data for pooled samples.

The NOAELs for immunotoxicity (IgM response) were 4800 ppm (equal to 727 mg/kg bw per day) in mice and 3500 ppm (equal to 229 mg/kg bw per day) in rats, the highest doses tested. The NOAELs for general toxicity were 4800 ppm (equal to 727 mg/kg bw per day) in mice, the highest dose tested, and 1000 ppm (equal to 68 mg/kg bw per day) in rats, based on body weight effects at 3500 ppm (Hoban, 2010a,b).

(c) Studies on metabolites

Metabolite IN-H8612 (ZA1963 metabolite 24 / R135305; 1,3-dihydro-3-oxoisobenzofuran-1-carboxylic acid)

IN-H8612 is formed in cereal grain treated with picoxystrobin; it has not been identified as a metabolite in rats.

IN-H8612 (purity 99%; batch P4) was of low acute oral toxicity to male and female Alpk:AP$_f$SD rats (LD_{50} > 2000 mg/kg bw) when administered in 1% carboxymethylcellulose. There were no signs of systemic toxicity (Twomey, 1999a).

In a 28-day toxicity study, IN-H8612 (purity 99%; batch P4) was administered to male and female Alpk:AP$_f$SD rats (five of each sex) at 0, 30, 500 or 1600 ppm in the diet. Overall mean intakes were 0, 3.5, 58 and 186 mg/kg bw per day for males and 0, 3.4, 58 and 182 mg/kg bw per day for females, respectively. Clinical observations, body weights and feed consumption were determined routinely. A functional observational battery and motor activity analyses were performed during week 4. At the end of the scheduled period, blood and urine samples were obtained, and the animals were

sacrificed and subjected to a full postmortem examination. Selected organs were weighed, and a wide range of tissues from control and top-dose animals and all tissues showing gross lesions from other groups were examined histopathologically. Individual animal data were not presented.

Achieved doses and homogeneity were confirmed by analyses.

All animals survived. There were no adverse effects on clinical signs of toxicity, body weight, feed consumption, ophthalmoscopy, functional observational battery, motor activity or haematology. Serum alanine aminotransferase activity was decreased significantly in 500 ppm (30%) and 1600 ppm (50%) females. Urine volume was increased in all treated groups, with a related reduction in specific gravity, but with no clear dose–response relationship. Increases in absolute testes weights, although statistically significant, were without a dose–response relationship and were secondary to slightly higher body weights. Reduced (15%) mean spleen weight in top-dose females persisted even when an outlier was removed, but were without any histopathological or haematological correlate. There were no adverse effects seen on macroscopic or microscopic examination of tissues. The findings at 1600 ppm are considered to be minimal in nature.

The NOAEL was 1600 ppm (equal to 182 mg/kg bw per day), the highest dose tested (Twomey, 1999b). (For comparison, the NOAEL in a 90-day rat toxicity study with picoxystrobin was 42 mg/kg bw per day, based on findings seen early on in the study.)

IN-H8612 (purity 99%; batch P4) was negative when tested for gene mutation at concentrations up to 5000 µg/plate in the presence and absence of metabolic activation in an Ames test with *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* WP2P *uvrA* (Callander, 1999a).

IN-H8612 (purity 99%; batch P4) produced positive results in a chromosomal aberration assay in human lymphocytes. The positive results were seen at a high concentration that produced a decrease in culture medium pH (by 1.1–2.2 pH units) and mild to moderate cytotoxicity. When the test material was neutralized to pH 7.4 with sodium hydroxide, cytotoxicity was reduced, and higher concentrations could be tested. In neutralized assays, a consistent pattern of increases in aberrations, particularly fragments, was seen at concentrations producing mild cytotoxicity. It is considered that the potential clastogenicity of IN-H8612 needs to be evaluated further (E. Jones, 1999).

Metabolite IN-QDY63 (ZA1963 metabolite 8 / R408509; 2-[2-(6-trifluoromethyl-2-pyridyloxymethyl)] benzoic acid)

IN-QDY63 is a major soil metabolite of picoxystrobin that has been found at low levels (< 5%) in rats and very occasionally in studies on plants.

IN-QDY63 (purity 97%; batch P5) was of moderate acute oral toxicity to male and female Alpk:AP$_f$SD rats (LD_{50} = 387 mg/kg bw) when administered in 1% carboxymethylcellulose (Twomey, 1999c).

In a 90-day toxicity study, IN-QDY63 (purity 97%; batch P5) was administered to male and female Alpk:AP$_f$SD rats (12 of each sex) at 0, 60, 180 or 600 ppm in the diet. Overall mean intakes were 0, 4.8, 14 and 48 mg/kg bw per day for males and 0, 5.2, 16 and 53 mg/kg bw per day for females, respectively. Clinical observations, body weights and feed consumption were determined routinely. A functional observational battery and motor activity analyses were performed during week 12. At the end of the scheduled period, blood and urine samples were obtained, and the animals were sacrificed and subjected to a full postmortem examination. Selected organs were weighed, and a wide range of tissues from control and top-dose animals and kidneys from all males were examined histopathologically. Individual animal data were not presented.

Achieved doses and homogeneity were confirmed by analyses.

All animals survived. There were no adverse effects on clinical signs of toxicity, feed consumption, ophthalmoscopy, functional observational battery, motor activity or haematology. Body weights from the top- and intermediate-dose male groups were lower (5%) than those of controls in the second half of the study. White blood cell count was reduced in the 60 and 600 ppm male groups, but not the 180 ppm group. Serum cholesterol levels were increased in all males, dose relatedly, but by a small amount (2.5, 2.7*, 2.8* and 2.9* mmol/l; * $P < 0.05$, *t*-test); top-dose groups had increases in plasma albumin levels. Relative liver weights were increased in top-dose males (7%) and females (9%), with absolute kidney weights increased in males (10%) and females (5%) from the 600 ppm groups. Renal tubular basophilia and interstitial cell infiltration were increased in top-dose males.

The NOAEL was 180 ppm (equal to 14 mg/kg bw per day), based on a number of findings at 600 ppm (equal to 48 mg/kg bw per day) (Twomey, 2000).

IN-QDY63 (purity 97%; batch P5) was negative when tested for gene mutation at concentrations up to 5000 µg/plate in the presence and absence of metabolic activation in an Ames test with *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* WP2P *uvrA* (Callander, 1999b). No mammalian cell genotoxicity studies were available on IN-QDY63.

IN-QDY63 was negative in an Ames test and has no structural alerts for genotoxicity (OECD QSAR [Quantitative Structure–Activity Relationship] Toolbox, version 2.1.2; Benigni/Bossa rules) and could be assigned to Cramer class 3, equating to a threshold of toxicological concern (TTC) of 90 µg/person per day (Kroes et al., 2004).

Phthalic acid

Phthalic acid is a widely used industrial chemical that has been identified as a plant metabolite of picoxystrobin. The toxicity of phthalic acid has been reviewed in a number of fora (e.g. USEPA, 1988; Integral, 2007). Phthalic acid is of low acute toxicity and is negative in an Ames test for mutagenicity (Zeiger et al., 1992; NTP, 2012) . It is not a teratogen and was not maternally toxic in rats at dose levels up to 5% in the diet (equal to 2981 mg/kg bw per day); the NOAEL for developmental toxicity was 2.5% (equal to 1763 mg/kg bw per day), based on decreased fetal weight and ossification at 5% (Ema et al., 1997). Phthalic anhydride, which hydrolyses to phthalic acid, is not carcinogenic in B6C3F1 mice or F344 rats at dose levels up to 1.5% (equal to 748 mg/kg bw per day) and 1.25% (equal to 3000 mg/kg bw per day), respectively (NCI, 1979). In the carcinogenicity studies, the NOAEL for toxicity was 1.5% (equal to 748 mg/kg bw per day) in rats, and the lowest-observed-adverse-effect level (LOAEL) was 6250 ppm (equal to 1562 mg/kg bw per day) in mice, based on lung and kidney pathology (NCI, 1979). The USEPA has set a chronic oral exposure reference dose of 2 mg/kg bw per day for phthalic anhydride, based on a LOAEL of 1562 mg/kg bw per day in a mouse carcinogenicity study, with the application of a nominal 1000-fold uncertainty factor (USEPA, 1988). The data indicate that phthalic acid is not a toxicologically relevant metabolite of picoxystrobin.

2-(2-Formylphenyl)-2-oxoacetic acid

2-(2-Formylphenyl)-2-oxoacetic acid is a plant metabolite of picoxystrobin that is not supported by any toxicological studies. It has not been identified in studies of metabolism in rats. An analysis using the profiling model in the OECD QSAR Toolbox (version 2.1.2) indicated that the aldehyde group on the molecule triggered an alert for potential "genotoxic carcinogenicity" (Benigni/Bossa rules). Until the genotoxicity of 2-(2-formylphenyl)-2-oxoacetic acid is investigated, the TTC-based exposure would be the lowest threshold of 0.15 µg/person per day (Kroes et al., 2004).

PAG3 (2-(2-Hydroxymethylphenyl)2-oxoacetic acid)

PAG3 is a plant metabolite of picoxystrobin that is not supported by any toxicological studies. It has not been identified in studies of metabolism in rats. An analysis using the profiling model in the

OECD QSAR Toolbox (version 2.1.2) indicated that PAG3 had no structural alerts for genotoxicity (Benigni/Bossa rules) and could be assigned to Cramer class III. Using a TTC approach, a compound in Cramer class III equates to an acceptable exposure of 90 µg/person per day (Kroes et al., 2004).

3. Observations in humans

No poisoning cases have been identified. No adverse findings have been reported in production plant workers (Calero, 2010; Bentley, 2012).

Comments

Biochemical aspects

Radiolabelled picoxystrobin administered by oral gavage is rapidly absorbed, with peak plasma ^{14}C levels seen at approximately 2 or 12 hours in rats administered 10 or 100 mg/kg bw, respectively. Picoxystrobin is well absorbed, with approximately 70% of the radioactivity from an oral dose of 100 mg/kg bw detected in bile and urine. Distribution is extensive, with peak radioactivity levels being detected in liver, pancreas, kidney and blood plasma. Excretion is predominantly via the bile and thence into faeces and is essentially complete within 120 hours for a dose of 100 mg/kg bw. Excretion in urine was greater in females (approximately 30%) than in males (approximately 20%). Picoxystrobin is extensively metabolized, with over 30 identified metabolites. Significant biotransformation reactions include ester hydrolysis, oxidation, *O*-demethylation and glucuronide conjugation.

Toxicological data

In the rat, picoxystrobin is of low acute oral and dermal toxicity (LD_{50}s > 2000 mg/kg bw), but is of high acute toxicity by inhalation (LC_{50} = 0.11 mg/l). In the rabbit, picoxystrobin is slightly irritating to the skin and moderately irritating to the eye, and it was not a skin sensitizer in a maximization test in guinea-pigs.

A consistent finding in animals exposed to picoxystrobin is reduced body weight gain, frequently associated with reduced feed consumption. Given the mode of pesticidal action on ATP production, the body weight effects might not be entirely secondary to the reduced feed consumption. Another common finding is increased liver weights.

In a 90-day dietary toxicity study in mice, the NOAEL was 800 ppm (equal to 137 mg/kg bw per day), based on increased relative liver weight (> 10%) and reduced body weight gains at 1600 ppm (equal to 291 mg/kg bw per day).

In a 90-day dietary toxicity study in rats, body weight gain was reduced from the first observation period (week 1), and terminal body weights were approximately 10% lower in the 1250 ppm (equal to 105 mg/kg bw per day) groups than in controls. Liver weight corrected for body weight was increased by more than 10% at 1250 ppm and by 5–8% at 500 ppm, but there were no associated pathological findings. The NOAEL was 500 ppm (equal to 42 mg/kg bw per day), based on the reductions in body weight gain and increased liver weights (> 10%) at 1250 ppm.

In a 90-day dietary toxicity study in dogs, reductions in body weights and feed consumption were seen from the first observation period (week 1) at 500 ppm (equal to 17 mg/kg bw per day), with a NOAEL of 250 ppm (equal to 8.5 mg/kg bw per day). In a 1-year dietary toxicity study in dogs, reddened gums, fluid faeces and thin appearance were seen, together with reductions in body weight and feed consumption, at 500 ppm (equal to 16 mg/kg bw per day). The NOAEL was 150 ppm (equal to 4.6 mg/kg bw per day). The Meeting concluded that an overall NOAEL for the dog studies was 8.5 mg/kg bw per day.

The chronic toxicity and carcinogenicity of picoxystrobin have been investigated in two studies in mice at dose levels up to 800 ppm or 4800 ppm and in two studies in rats at dose levels up to 750 ppm or 3500 ppm. In the first mouse carcinogenicity study, slight, but not adverse, reductions in body weight (approximately 5%) and increases in liver weights (approximately 10%) were seen at

800 ppm (equal to 109 mg/kg bw per day), the highest dose tested. In the second mouse carcinogenicity study, survival was significantly increased in males receiving 2400 or 4800 ppm. Pathological changes seen in the liver in the 4800 ppm groups were macroscopic nodules, microscopic foci of alteration and a significant increase in total hepatocellular tumours in males and centrilobular hepatocyte hypertrophy in females. The tumours were seen predominantly in males surviving to the end of the study, and additional statistical analyses indicated that the increases were related to the increased survival in these mice. The NOAEL for toxicity in mice was 600 ppm (equal to 71 mg/kg bw per day), based on increased liver weights (> 10%) in both sexes at 2400 ppm (equal to 293 mg/kg bw per day). The NOAEL for carcinogenicity was 4800 ppm (equal to 585 mg/kg bw per day), the highest dose tested, as the increase in liver tumours in males at 2400 and 4800 ppm is considered to be secondary to increased survival in these groups and therefore not relevant to the risk assessment of picoxystrobin.

In the first rat chronic toxicity and carcinogenicity study, Alpk (Wistar-derived) male rats receiving 750 ppm had increased survival and increased incidences of large granular lymphocyte leukaemia. The incidence of leukaemia was outside the test facility's historical control range and was still statistically significant when corrected for the increased survival. The leukaemia incidence was of marginal statistical significance, it is a spontaneous finding in this strain of rat, there were no associated pathological changes in other organs (e.g. the spleen) and the finding was not duplicated in a second study that employed higher dose levels; therefore, the Meeting concluded that these leukaemias were incidental findings. In the first study, the NOAEL for toxicity was 200 ppm (equal to 12 mg/kg bw per day), based on reduced body weight gain and kidney weights at 750 ppm (equal to 46 mg/kg bw per day). In the second rat chronic toxicity and carcinogenicity study, in Crl:CD(SD) rats (Sprague-Dawley derived), survival was increased in the picoxystrobin groups, with over twice as many rats from the 3500 ppm groups surviving to the end of the study. Reductions in body weight gain, feed consumption and feed conversion efficiency were seen during the 1st year of the study in the 3500 ppm groups. Liver weight relative to body weight was increased by more than 10% at both interim and terminal kills in the 3500 ppm groups. Testes weights were increased in top-dose rats at the interim kill. Statistically significant increases in the incidences of interstitial cell hyperplasia and benign adenoma in the testes were observed in male rats at 3500 ppm. Although the majority of adenomas and hyperplasia occurred in terminal or near-terminal animals, the results were still statistically significant when corrected for the increased survival, and the Meeting considered it likely that the increases in testicular interstitial cell adenoma and hyperplasia in the 3500 ppm males were related to exposure to the test substance. The NOAEL for toxicity in rats was 1000 ppm (equal to 45 mg/kg bw per day), based on testicular interstitial cell hyperplasia and benign adenoma, reduced body weights and increased relative liver weights at 3500 ppm (equal to 162 mg/kg bw per day).

The Meeting concluded that picoxystrobin is not carcinogenic to mice or rats.

Picoxystrobin has been tested in an adequate range of genotoxicity studies. No evidence of genotoxicity was seen, other than a weak response in a mouse lymphoma mammalian cell gene mutation assay with metabolic activation.

The Meeting concluded that picoxystrobin is unlikely to be genotoxic.

Based on the lack of genotoxicity and the absence of carcinogenicity in rats and mice, the Meeting concluded that picoxystrobin is unlikely to pose a carcinogenic risk to humans.

Two multigeneration reproductive toxicity studies in rats have been performed with picoxystrobin at dose levels up to 750 ppm or 2500 ppm. In the first study, the NOAEL for reproductive toxicity was 750 ppm (equal to 78 mg/kg bw per day), the highest dose tested. The NOAEL for parental and offspring toxicity was 200 ppm (equal to 21 mg/kg bw per day), based on reductions in body weight gain at 750 ppm. In the second study, the NOAEL for reproductive toxicity was 2500 ppm (equal to 130 mg/kg bw per day), the highest dose tested. The NOAEL for parental and offspring toxicity was 1000 ppm (equal to 52 mg/kg bw per day), based on body weight deficits and thymic atrophy at 2500 ppm in parental animals and pups.

The developmental toxicity of picoxystrobin has been assessed in rats and rabbits. In rats, misaligned 5th sternebrae were present in 1.5% of fetuses in the 100 mg/kg bw per day group, above the cited historical control range of 0–1.4%. The NOAEL for developmental toxicity was 30 mg/kg bw per day, based on an increased incidence of misaligned 5th sternebrae at 100 mg/kg bw per day. The NOAEL for maternal toxicity was 30 mg/kg bw per day, based on body weight deficits and reduced feed consumption during the dosing period at 100 mg/kg bw per day.

In rabbits, the mean number of fetuses per litter was reduced at 100 mg/kg bw per day, but this appeared to be related to increased preimplantation losses, which occurred before the start of administration of picoxystrobin. Increased incidences of skeletal anomalies were seen in the 100 mg/kg bw per day group. The NOAEL for developmental toxicity was 25 mg/kg bw per day, based on an increase in skeletal anomalies at 100 mg/kg bw per day. The NOAEL for maternal toxicity was 25 mg/kg bw per day, based on body weight deficits and reduced feed consumption during the dosing period at 100 mg/kg bw per day.

The Meeting concluded that picoxystrobin is not teratogenic in rats or rabbits.

In an acute neurotoxicity study in rats, there was no evidence of neuropathy at 2000 mg/kg bw, the highest dose tested. The NOAEL for toxicity was less than 200 mg/kg bw, based on dose-related, transient decreases in motor activity and in body weight at all dose levels. A BMD evaluation indicated that a derived NOAEL in the acute neurotoxicity study was likely to be much lower than 200 mg/kg bw. There was no evidence of neurotoxicity in a 90-day neurotoxicity study in rats at dose levels up to 3500 ppm (equal to 207 mg/kg bw per day), the highest dose tested.

In a 28-day dietary immunotoxicity study in rats and mice, no effects on IgM response to sRBCs were observed at the highest doses tested, 4800 ppm (equal to 727 mg/kg bw per day) in mice or 3500 ppm (equal to 229 mg/kg bw per day) in rats.

The plant metabolite IN-H8612 (1,3-dihydro-3-oxoisobenzofuran-1-carboxylic acid) was of low acute oral toxicity to rats (LD_{50} > 2000 mg/kg bw). In a 28-day toxicity study in rats, the NOAEL was 1600 ppm (equal to 182 mg/kg bw per day), the highest dose tested. IN-H8612 was negative in an Ames test for bacterial gene mutation and produced equivocal results at high concentrations in a chromosomal aberration assay in human lymphocytes. On the basis of the limited in vivo data available, the toxicological potency of IN-H8612 is similar to or lower than that of picoxystrobin, but additional data are required to resolve its potential to induce chromosomal aberrations in vivo. The Meeting was unable to conclude on the genotoxic potential of IN-H8612. The international estimated daily intake (IEDI) was above 0.15 µg/person per day, the TTC for a compound with evidence of genotoxicity. The Meeting was unable to conclude on the toxicological relevance of estimated intakes of IN-H8612.

The soil metabolite IN-QDY63 (2-[2-(6-trifluoromethyl-2-pyridyloxymethyl)] benzoic acid) was of moderate acute oral toxicity to rats (LD_{50} = 387 mg/kg bw). In a 90-day dietary toxicity study in rats, the NOAEL was 180 ppm (equal to 14 mg/kg bw per day), based on increased kidney weights and renal tubule pathological changes at 600 ppm (equal to 48 mg/kg bw per day). IN-QDY63 was negative in an Ames test for bacterial gene mutation. The limited data available indicate that the toxicity of IN-QDY63 is quantitatively similar to or greater than that of picoxystrobin. The estimated IEDI for IN-QDY63 was 9.7 µg/person per day, below the applicable TTC of 90 µg/person per day. The Meeting concluded that IN-QDY63 was not of toxicological concern at the estimated dietary intake levels.

Three other plant metabolites of picoxystrobin were considered:

- Phthalic acid is a widely used industrial chemical. It is not genotoxic and is not a developmental toxicant in rats (NOAEL = 1763 mg/kg bw per day). Phthalic anhydride, which hydrolyses to phthalic acid, is not carcinogenic in mice or rats, with NOAELs equal to or above 748 mg/kg bw per day. The Meeting concluded that phthalic acid is not a toxicologically relevant metabolite of picoxystrobin.

- 2-(2-Formylphenyl)-2-oxoacetic acid is not supported by any toxicological studies, but a structural alert for genotoxicity was identified. The IEDI was above 0.15 µg/person per day, the TTC for a compound with a structural alert for genotoxicity. The Meeting was unable to conclude on the toxicological relevance of estimated intakes of 2-(2-formylphenyl)-2-oxoacetic acid.
- 2-(2-Hydroxymethylphenyl)-2-oxoacetic acid (PAG3) is not supported by any toxicological studies, and no structural alerts for genotoxicity were identified. The estimated IEDI for PAG3 was 16.4 µg/person per day, below the applicable TTC of 90 µg/person per day. The Meeting concluded that PAG3 was not of toxicological concern at the estimated dietary intake levels.

Medical surveillance of production plant workers has not identified any cases of occupational illness related to picoxystrobin. There are no reports of poisoning cases with picoxystrobin.

The Meeting concluded that the existing database on picoxystrobin was adequate to characterize the potential hazards to fetuses, infants and children.

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) of 0–0.09 mg/kg bw on the basis of the overall NOAEL of 8.5 mg/kg bw per day in the 90-day and 1-year dog studies, based on body weight loss, reduced feed consumption and clinical signs at 16 mg/kg bw per day. A safety factor of 100 was applied.

The Meeting established an acute reference dose (ARfD) of 0.09 mg/kg bw on the basis of the overall NOAEL of 8.5 mg/kg bw per day in the 90-day and 1-year dog studies, based on body weight loss and reduced feed consumption at the beginning of the study at 16 mg/kg bw per day. A safety factor of 100 was applied. This value is supported by a BMD analysis of the motor activity changes seen at the lowest dose in the acute neurotoxicity study. The Meeting noted that this ARfD is possibly conservative and that it might be possible to refine it.

Picoxystrobin's mode of fungicidal activity is to block mitochondrial electron transport, reducing ATP production and inhibiting cellular respiration; this could result in impaired body weight gains. In the absence of any information on the mode of toxicological action for reductions in body weight and body weight gain, these effects were considered as adverse and relevant for the setting of guidance values.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen-month studies of toxicity and carcinogenicity[a,b]	Toxicity	600 ppm, equal to 71 mg/kg bw per day	2400 ppm, equal to 293 mg/kg bw per day
		Carcinogenicity	4800 ppm, equal to 585 mg/kg bw per day[c]	—

Species	Study	Effect	NOAEL	LOAEL
Rat	Acute neurotoxicity study[d]	Toxicity	—	200 mg/kg bw per day[e]
	Ninety-day study of toxicity[a]	Toxicity	500 ppm, equal to 42 mg/kg bw per day	1250 ppm, equal to 105 mg/kg bw per day
	Two-year study of toxicity and carcinogenicity[a]	Toxicity	1000 ppm, equal to 45 mg/kg bw per day	3500 ppm, equal to 162 mg/kg bw per day
		Carcinogenicity	3500 ppm, equal to 162 mg/kg bw per day[c]	—
	Multigeneration study of reproductive toxicity[a]	Reproductive toxicity	2500 ppm, equal to 130 mg/kg bw per day[c]	—
		Parental toxicity	1000 ppm, equal to 52 mg/kg bw per day	2500 ppm, equal to 130 mg/kg bw per day
		Offspring toxicity	1000 ppm, equal to 52 mg/kg bw per day	2500 ppm, equal to 130 mg/kg bw per day
	Developmental toxicity study[d]	Maternal toxicity	30 mg/kg bw per day	100 mg/kg bw per day
		Embryo and fetal toxicity	30 mg/kg bw per day	100 mg/kg bw per day
Rabbit	Developmental toxicity study[d]	Maternal toxicity	25 mg/kg bw per day	100 mg/kg bw per day
		Embryo and fetal toxicity	25 mg/kg bw per day	100 mg/kg bw per day
Dog	Ninety-day and 1-year studies of toxicity[a,b]	Toxicity	250 ppm, equal to 8.5 mg/kg bw per day	500 ppm, equal to 16 mg/kg bw per day

[a] Dietary administration.
[b] Two or more studies combined.
[c] Highest dose tested.
[d] Gavage administration.
[e] Lowest dose tested.

Estimate of acceptable daily intake for humans

0–0.09 mg/kg bw

Estimate of acute reference dose

0.09 mg/kg bw

Information that would be useful for the continued evaluation of the compound

Additional data on the genotoxicity of the plant metabolites IN-H8612 and 2-(2-formylphenyl)-2-oxoacetic acid

Results from epidemiological, occupational health and other such observational studies of human exposure

Critical end-points for setting guidance values for exposure to picoxystrobin

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	75% in 48 h, based on urinary and biliary excretion
Distribution	Extensive; highest levels in liver and kidneys
Potential for accumulation	No evidence for accumulation
Rate and extent of excretion	> 95% within 5 days (low dose); mainly bile and faeces
Metabolism in animals	Extensive, with over 30 identified metabolites
Toxicologically significant compounds in animals, plants and the environment	Picoxystrobin, IN-H8612 and 2-(2-formylphenyl)-2-oxoacetic acid (plant metabolites) and IN-QDY63 (soil metabolite)
Acute toxicity	
Rat, LD_{50}, oral	> 5000 mg/kg bw
Rat, LD_{50}, dermal	> 2000 mg/kg bw
Rat, LC_{50}, inhalation	0.11 mg/l (4 h, nose-only)
Rabbit, dermal irritation	Slightly irritating
Rabbit, ocular irritation	Moderately irritating
Dermal sensitization	Not a sensitizer (Magnusson & Kligman test in guinea-pigs)
Short-term studies of toxicity	
Target/critical effect	Reduced body weight and feed consumption; clinical signs (dog)
Lowest relevant oral NOAEL	8.5 mg/kg bw per day (90-day and 1-year dog combined)
Lowest relevant dermal NOAEL	1000 mg/kg bw per day (28-day rat)
Lowest relevant inhalation NOAEC	0.025 mg/l (28-day rat)
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Reduced body weight and feed consumption; liver (mouse); testes, interstitial cell hyperplasia and adenoma (rat)
Lowest relevant NOAEL	45 mg/kg bw per day (2-year rat)
Carcinogenicity	Not carcinogenic
Genotoxicity	
	Unlikely to be genotoxic
Reproductive toxicity	
Reproduction target/critical effect	Reduced body weight gain in parents and pups
Lowest relevant reproductive NOAEL	130 mg/kg bw per day (highest dose tested)
Lowest relevant parental NOAEL	52 mg/kg bw per day
Lowest relevant offspring NOAEL	52 mg/kg bw per day
Developmental toxicity	
Developmental target/critical effect	Reduced maternal and fetal body weight; skeletal anomalies
Lowest relevant maternal NOAEL	25 mg/kg bw per day (rabbit)
Lowest relevant embryo/fetal NOAEL	25 mg/kg bw per day (rabbit)
Neurotoxicity	
Acute neurotoxicity	Reduced motor activity, reduced body weight: LOAEL 200 mg/kg bw (rat)

Subchronic (90-day) neurotoxicity	Not neurotoxic: NOAEL 207 mg/kg bw per day (highest dose tested) (rat)
Immunotoxicity	
Twenty-eight-day study	Not immunotoxic in mice or rats: NOAEL 229 mg/kg bw per day (highest dose tested) (rats)
Other toxicological studies	
Metabolite IN-H8612	Oral LD_{50} > 2000 mg/kg bw (rat)
	28-day toxicity study in rats: NOAEL 182 mg/kg bw per day
	Not mutagenic in bacteria; equivocal results for chromosomal aberrations in vitro
Metabolite IN-QDY63	Oral LD_{50} 387 mg/kg bw (rat)
	90-day toxicity study in rats: NOAEL 14 mg/kg bw per day
	Not mutagenic in bacteria
Medical data	
	No reports of poisonings or adverse effects in production plant workers

Summary

	Value	Study	Safety factor
ADI	0–0.09 mg/kg bw	90-day and 1-year studies (dog)	100
ARfD	0.09 mg/kg bw	90-day and 1-year studies (dog)	100

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SEDAXANE

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Explanation

Sedaxane is the common name that has been provisionally approved by the International Organization for Standardization (ISO) for mixtures of two *cis* isomers, 2′-[(1*RS*,2*RS*)-1,1′-bicycloprop-2-yl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxanilide, and two *trans* isomers, 2′-[(1*RS*,2*SR*)-1,1′-bicycloprop-2-yl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxanilide (International Union of Pure and Applied Chemistry), for which the Chemical Abstracts Service number is 874967-67-6. *Trans* and *cis* isomers of sedaxane show comparable toxicological profiles, and both are fungicidally active. The definition of the active ingredient is a mixture of the two isomers in ratios of

a minimum 810 g/kg *trans* isomer and a maximum 150 g/kg *cis* isomer. The nominal (typical) content is approximately 980 g/kg. Sedaxane contains approximately 81–85% of the *trans* isomers and approximately 10–15% of the *cis* isomers.

Sedaxane is a new broad-spectrum seed-applied fungicide belonging to the chemical class of pyrazole-carboxamides. The pesticidal mode of action of this group of fungicides is inhibition of succinate dehydrogenase, which is a functional part of the mitochondrial electron transport chain and oxidative phosphorylation involved in the tricarboxylic acid cycle. It is efficient in the control of a wide range of fungal pathogens, including *Microdochium nivale*, smut (*Ustilago tritici*), stink bunt (*Tilletia caries*), loose smut (*Ustilago nuda*), head smut (*Sphacelotheca reiliana*), Asian soya bean rust (*Phakopsora pachyrhizi*) and *Rhizoctonia* spp.

Sedaxane is being reviewed for the first time by the Joint FAO/WHO Expert Meeting on Pesticide Residues at the request of the Codex Committee on Pesticide Residues. All critical studies complied with good laboratory practice.

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

The mammalian metabolism of sedaxane has been assessed in studies investigating its absorption, distribution, metabolism and excretion in rats. In a biotransformation study, the nature of the metabolites formed was determined both qualitatively and quantitatively. The fate was investigated following administration of both single and multiple doses. Biliary elimination studies were conducted with [^{14}C-pyrazole]sedaxane and [^{14}C-phenyl]sedaxane. As evidence suggested that cleavage of the sedaxane molecule during biotransformation was a relatively minor process, a single radiolabelled form was used in the remaining metabolism studies. In these studies, radiolabelled material comprising an approximate 6:1 mixture of the *trans* and *cis* isomers was used, which is therefore similar to the definition ratio of 5.77.

The structure and positions of the ^{14}C radiolabel in the two radiolabelled forms of sedaxane used in the metabolism studies are shown in Figure 1.

Figure 1. Structures of the radiolabelled molecules

O HF$_2$C NH N N * CH$_3$

[Pyrazole-5-^{14}C]sedaxane

* O HF$_2$C NH N N CH$_3$

[Phenyl-U-^{14}C]sedaxane

(a) Absorption

Oral

A single oral dose of [pyrazole-5-^{14}C]sedaxane (lot nos CL-LXII-15 for low dose, CL-LXII-14 for high dose; purity 97.0–99.2%; ratios of isomers 84.6% *trans* to 15.4% *cis* for low dose and 85.9% *trans* to 14.1% *cis* for high dose) was administered by gavage to eight male and eight female bile duct–cannulated Han Wistar rats at a dose level of 1 or 80 mg/kg body weight (bw). The excretion of radioactivity was measured over 2 days. After this period, the rats were killed, and

residual radioactivity was measured in blood, plasma, the gastrointestinal tract (and contents) and the remaining carcass (Shaw, 2009c). In addition, a single oral dose of [phenyl-U-^{14}C]sedaxane (lot nos RDR-III-25 for low dose, RDR-III-24 for high dose; purity 98.6–99.1%; ratios of isomers 86.0% *trans* to 14.0% *cis* for low dose and 85.6% *trans* to 14.4% *cis* for high dose) was administered in the same manner as for [pyrazole-5-^{14}C]sedaxane (Shaw, 2009d).

The major route of elimination of a single oral dose of 1 mg/kg bw of both [pyrazole-5-^{14}C]sedaxane and [phenyl-U-^{14}C]sedaxane was via the bile, with means of 78.6–81.1% of the dose recovered in male and female bile over 2 days post-dosing. Urinary excretion accounted for means of 6.5–8.1% of the dose in males and females. Faecal excretion accounted for means of 4.7–8.6% in males and females by the end of the sampling period (Tables 1 and 2). Excretion of both [pyrazole-5-^{14}C]sedaxane and [phenyl-U-^{14}C]sedaxane at the low dose was rapid, with most of the administered radioactivity excreted by 24 hours after dosing (for pyrazole-5-^{14}C, means of approximately 91.4% and 90.5% in males and females, respectively; for phenyl-U-^{14}C, means of 93.6% and 94.5% in males and females, respectively). Means of 87.4% and 87.9% of the administered dose of [pyrazole-5-^{14}C]sedaxane and 89.1% and 87.5% of the administered dose of [phenyl-U-^{14}C]sedaxane were absorbed in males and females, respectively, as calculated from the radioactivity eliminated in urine, daily cage wash and bile over 2 days, together with that present in the residual carcass (Table 1). The total mean per cent recoveries of administered radioactivity including excreta and residual carcasses following oral gavage dosing at 1 mg/kg bw were 94.1% for males and 92.6% for females treated with [pyrazole-5-^{14}C]sedaxane and 94.9% for males and 96.1% for females treated with [phenyl-U-^{14}C]sedaxane, respectively (Table 2).

Table 1. Absorption of radioactivity after oral administration of [pyrazole-5-^{14}C]sedaxane and [phenyl-U-^{14}C]sedaxane

	Absorption (% of radioactive dose)							
	[Pyrazole-5-^{14}C]sedaxane				[Phenyl-U-^{14}C]sedaxane			
	1 mg/kg bw		80 mg/kg bw		1 mg/kg bw		80 mg/kg bw	
	Males (*n* = 4)	Females (*n* = 4)	Males (*n* = 4)	Females (*n* = 3)	Males (*n* = 4)	Females (*n* = 4)	Males (*n* = 4)	Females (*n* = 3)
Urine	6.7	6.9	5.9	10.2	6.5	8.1	6.7	5.3
Bile	79.0	79.4	81.8	81.2	81.1	78.6	85.3	81.0
Cage wash	1.4	1.5	1.3	0.9	1.2	0.7	1.6	0.7
Carcass	0.3	0.1	0.5	0.2	0.3	0.2	0.3	0.1
Total absorption	87.4	87.9	89.5	92.5	89.1	87.5	93.9	87.1

From Shaw (2009c,d)

Following a single oral dose of 80 mg/kg bw of [pyrazole-5-^{14}C]sedaxane or [phenyl-U-^{14}C]sedaxane, the major route of elimination was similarly via the bile in both sexes, with means of 81.0–85.3% of the administered radioactivity recovered in bile over 2 days post-dosing in males and females. Urinary excretion of [pyrazole-5-^{14}C]sedaxane accounted for means of 5.9% and 10.2% of the dose in males and females, respectively. Urinary excretion of [phenyl-U-^{14}C]sedaxane accounted for means of 6.7% and 5.3% of the dose in males and females, respectively. Faecal elimination of [pyrazole-5-^{14}C]sedaxane accounted for means of 7.1% and 3.3% of the administered dose in males and females, respectively. Faecal elimination of [phenyl-U-^{14}C]sedaxane accounted for means of 4.4% and 10.6% of the administered dose in males and females, respectively (Tables 1 and 2). Excretion of both [pyrazole-5-^{14}C]sedaxane and [phenyl-U-^{14}C]sedaxane at the high dose was rapid, with the majority of the administered radioactivity excreted by 24 hours post-dosing (for pyrazole-5-^{14}C, means of 92.0% and 93.1% in males and females, respectively; for phenyl-U-^{14}C, means of 96.1% and 95.7% in males and females, respectively). Group means of 89.5% and 92.5% of the administered

dose of [pyrazole-5-^{14}C]sedaxane and 93.9% and 87.1% of the administered dose of [phenyl-U-^{14}C]sedaxane were absorbed in males and females, respectively, as calculated from the radioactivity eliminated in urine, daily cage wash and bile over 2 days, together with that present in the residual carcass (Table 1). The total mean per cent recoveries of administered radioactivity including excreta and residues in carcasses following oral gavage dosing at 80 mg/kg bw were 96.6% for males and 95.9% for females with [pyrazole-5-^{14}C]sedaxane and 98.4% for males and 97.7% for females with [phenyl-U-^{14}C]sedaxane (Table 2).

Table 2. Recovery of radioactivity in excreta and bile after administration of a single oral dose of [pyrazole-5-^{14}C]sedaxane and [phenyl-U-^{14}C]sedaxane to bile duct–cannulated rats

		Group mean excretion (% of radioactive dose recovered)[a]							
		[Pyrazole-5-^{14}C]sedaxane				[Phenyl-U-^{14}C]sedaxane			
		1 mg/kg bw		80 mg/kg bw		1 mg/kg bw		80 mg/kg bw	
		Males (n = 4)	Females (n = 4)	Males (n = 4)	Females (n = 4)	Males (n = 4)	Females (n = 4)	Males (n = 4)	Females (n = 4)
Urine	0–24 h	6.5	6.7	5.6	10.0	6.4	7.9	6.4	5.1
	24–48 h	0.2	0.1	0.3	0.2	0.2	0.2	0.4	0.2
	Subtotal	6.7	6.9	5.9	10.2	6.5	8.1	6.7	5.3
Faeces	0–24 h	6.3	4.6	6.5	2.9	5.5	8.3	4.0	10.0
	24–48 h	0.4	0.2	0.6	0.4	0.2	0.3	0.4	0.5
	Subtotal	6.6	4.7	7.1	3.3	5.7	8.6	4.4	10.6
Bile	0–0.5 h	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	0.5–1 h	< 0.2	3.2	0.8	0.4	< 0.3	< 1.8	0.6	< 0.5
	1–2 h	15.6	16.8	5.7	4.1	14.0	15.4	5.1	4.7
	2–4 h	21.0	21.6	11.3	10.6	24.9	18.2	9.4	9.9
	4–8 h	26.7	24.8	23.7	20.8	29.1	24.7	24.1	21.1
	8–24 h	15.2	12.9	38.5	44.3	12.7	17.9	45.2	44.0
	24–48 h	0.4	0.2	1.9	1.0	0.3	0.6	0.9	1.0
	Subtotal	79.0	79.4	81.8	81.2	81.1	78.6	85.3	81.0
Cage wash		1.4	1.5	1.3	0.9	1.2	0.7	1.6	0.7
Gastrointestinal tract + contents		< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1	< 0.1
Carcass		0.3	0.1	0.5	0.2	0.3	0.2	0.3	0.1
Total recovery		94.1	92.6	96.6	95.9	94.9	96.1	98.4	97.7

From Shaw (2009c)

[a] Total and subtotal values may not add up exactly due to rounding of values.

In conclusion, irrespective of dose level or sex, a single oral dose of [pyrazole-5-^{14}C]sedaxane or [phenyl-U-^{14}C]sedaxane at 1 or 80 mg/kg bw was extensively absorbed, representing at least 87% of the dose, and was rapidly and extensively eliminated, predominantly via the bile. By 2 days after dosing, carcass residues represented 0.5% of the dose or less for [pyrazole-5-^{14}C]sedaxane and 0.3% of the dose or less for [phenyl-U-^{14}C]sedaxane (Shaw, 2009c,d).

Dermal

Dermal absorption factors for risk assessment were derived from three studies utilizing [pyrazole-5-^{14}C]sedaxane in a flowable concentrate for seed treatment formulation, A16148C. These involved an in vivo rat study (Read & Sweeney, 2009) and in vitro studies with epidermal membranes from rats or humans (Davies, 2009a,b). It has been established in numerous scientific studies that rat skin is more permeable than human skin in the course of typical exposures. Examples where this has been studied in vitro using pesticides can be found in Scott & Corrigan (1990), Scott et al. (1991), Ramsey et al. (1994), Van de Sandt et al. (2000), Cnubben et al. (2002) and Van Ravenzwaay &

Leibold (2004). To account for this difference and to more accurately estimate the dermal absorption in humans, European Union and international guidance utilizes the comparative ratio of rat and human in vitro dermal absorption results to correct the in vivo rat values (EC, 2004; IPCS, 2006).

(b) Pharmacokinetics

The kinetics of [pyrazole-5-^{14}C]sedaxane (lot nos CL-LXII-15 for low dose, CL-LXII-14 for high dose; purity 97.0–99.2%; ratios of isomers 84.6% *trans* to 15.4% *cis* for low dose and 85.9% *trans* to 14.1% *cis* for high dose) was examined using Han Wistar rats given a single oral dose of 1 or 80 mg/kg bw. In addition, excreta samples were collected for total radioactivity analysis (Shaw, 2009b).

Following administration of a single dose of [pyrazole-5-^{14}C]sedaxane at 1 mg/kg bw to male and female rats, the observed maximum concentration (C_{max}) of radioactivity in plasma was 0.11 µg equivalent (Eq) per millilitre in both males and females, with the time taken to reach C_{max} (T_{max}) ranging between 1 and 1.5 hours after dosing. The estimated terminal half-life ($t_{½}$ term.) was 22.65 hours and 24.85 hours in males and females, respectively, and the area under the plasma concentration–time curve (AUC_{0-t}) was 1.663 µg Eq·h/ml in males and 1.625 µg Eq·h/ml in females. Only low residues of radioactivity were detectable 72 hours after dosing (Table 3).

Following administration of a single oral dose of [pyrazole-5-^{14}C]sedaxane at 80 mg/kg bw to male and female rats, the observed mean peak concentrations of radioactivity in plasma (C_{max}) were 10.6 and 12.4 µg Eq/ml in males and females, respectively, with T_{max} ranging between 5 and 6 hours after dosing. The estimated $t_{½}$ term. was 28.76 hours and 23.29 hours in males and females, respectively, and the AUC_{0-t} was 233.5 µg Eq·h/ml in males and 192.2 µg Eq·h/ml in females (Table 3). Residues of radioactivity were still detectable 72 hours after dosing.

Systemic exposure to the total radioactivity (AUC) associated with [pyrazole-5-^{14}C]sedaxane in plasma tended to be slightly greater than that in whole blood in both sexes and at both dose levels. This suggests that total radioactivity was relatively evenly distributed between plasma and the cellular component of the blood at both dose levels. Pharmacokinetic parameters of total radioactivity in blood were therefore not appreciably different from those in plasma either between the sexes or between the dose levels.

A comparison between the low and high dose levels showed that increasing the dose of [pyrazole-5-^{14}C]sedaxane from 1 to 80 mg/kg bw produced a 140- and 120-fold increase in plasma AUC in males and females, respectively. However, because of the observed variability in the concentrations for individual rats, this was considered not to be significant.

Table 3. Summary of blood and plasma toxicokinetic parameters following administration of [pyrazole-5-^{14}C]sedaxane to rats

Toxicokinetic parameter	Blood				Plasma			
	1 mg/kg bw		80 mg/kg bw		1 mg/kg bw		80 mg/kg bw	
	Males	Females	Males	Females	Males	Females	Males	Females
C_{max} (µg Eq/ml)	0.071	0.075	9.035	9.597	0.106	0.110	10.572	12.432
T_{max} (h)	1	1.5	5	4	1	1.5	5	6
AUC_{0-t} (µg Eq·h/ml)	1.464	1.301	196.9	158.2	1.663	1.625	233.5	192.2
$t_{½}$ (h)	39.86	33.92	31.61	20.71	22.65	24.85	28.76	23.29

From Shaw (2009b)
AUC, area under the plasma–concentration time curve; C_{max}, peak plasma concentration; $t_{½}$, half-life; T_{max}, time to reach C_{max}

Following administration of a single oral dose of [pyrazole-5-^{14}C]sedaxane to male and female rats, the mean peak plasma concentrations were reached after approximately 1 hour at the low dose level and 5 hours at the high dose level. Systemic exposure was similar for males and females at both dose levels (Shaw, 2009b).

(c) Distribution

Single dose

A single oral dose of [pyrazole-5-^{14}C]sedaxane (lot nos CL-LXII-15 for low dose, CL-LXII-14 for high dose; purity 97.0–99.2%; ratios of isomers 84.6% *trans* to 15.4% *cis* for low dose and 85.9% *trans* to 14.1% *cis* for high dose) was administered by gavage to 30 male and 30 female rats (Han Wistar) at a nominal dose level of 1 or 80 mg/kg bw. Groups of three rats were killed at various times post-dosing, and selected organs, tissues and body fluids were collected for total radioactivity analysis. The terminal half-life for each tissue depletion was also estimated.

Radioactivity from a single oral dose of 1 mg/kg bw was widely distributed to the tissues in both sexes (Table 4). Peak mean tissue concentrations were attained at the first sampling time. The highest mean concentration of radioactivity was present in the liver of both sexes, with liver and kidney concentrations remaining above plasma concentrations throughout the course of the experiment. High concentrations were also present in the pancreas, adrenals and adipose tissue until approximately 8 hours post-dosing. Thereafter, all but liver and kidney levels declined to concentrations close to or below the limit of reliable measurement.

Radioactivity from a single oral dose of 80 mg/kg bw was widely distributed to the tissues in both sexes (Table 5). Maximum mean tissue concentrations were observed at the first sampling time (5 hours post-dosing in both sexes). The highest mean concentrations of radioactivity were present in the liver and adipose tissue of both sexes. However, the levels in fat declined rapidly and by 48 hours were below plasma concentrations in both sexes. High concentrations were also present in the pancreas, adrenals and thyroid up to approximately 24 hours post-dosing. Thereafter, these concentrations declined to values close to or below mean plasma concentrations. In both sexes, the liver and kidney concentrations remained above mean plasma concentrations throughout the course of the experiment. By 96 hours after dosing, total radioactivity had declined extensively in all other tissues, with mean concentrations close to or below the limit of reliable measurement.

Tissue distribution was generally similar in males and females at both dose levels. The half-lives of tissue depletion of total radioactivity were variable, the shortest and longest estimates occurring in brain (0.1–0.2 day) and thyroid (2.0–3.2 days), respectively (Table 6).

In both male and female rats, the tissue distribution of radioactivity was similar and extensive following a single oral dose of 1 or 80 mg [pyrazole-5-^{14}C]sedaxane per kilogram body weight. Tissue concentrations of radioactivity were highest at the first sampling time (1–1.5 hours for low dose and 5 hours for high dose) and progressively declined thereafter, with elimination half-lives of between 0.1 and 3.2 days. Most mean tissue concentrations were close to or below the limit of reliable measurement by 96 hours post-dosing, when mean total tissue and carcass residues accounted for less than 0.8% of the dose. The high levels of radioactivity in the gastrointestinal tract and its contents throughout the 96-hour time course were consistent with the established biliary excretion and faecal elimination of sedaxane metabolites (Shaw, 2009e).

Repeated dose

Thirty-three male Han Wistar rats were given up to 14 consecutive daily oral doses of [pyrazole-5-^{14}C]sedaxane (lot no. RDR-II-75; purity 98.6%; ratio of isomers 84.2% *trans* to 13.6% *cis*) at 1 mg/kg bw per day. At predetermined intervals during dosing and following the cessation of dosing, groups of rats were killed for the removal of selected tissues or organs to determine the extent of accumulation of radioactivity in tissues and the remaining carcasses and its subsequent elimination. Additionally, the excretion of radioactivity in urine and faeces was monitored in one group of rats for a period of 24 hours following the 1st and 14th doses.

Table 4. Distribution of radioactivity in tissues/organs 1 (males), 1.5 (females), 8, 24, 48 and 96 hours after administration of [pyrazole-5-^{14}C]sedaxane to rats at a dose level of 1 mg/kg bw

	Group mean tissue residues (µg Eq/g or ml)									
	Males					Females				
	1 h	8 h	24 h	48 h	96 h	1.5 h	8 h	24 h	48 h	96 h
Adrenals	0.245	0.084	0.016	< 0.006	< 0.003	0.312	< 0.033	0.008	0.006	< 0.003
Bone mineral	0.024	0.029	0.004	< 0.002	< 0.001	0.021	0.011	0.003	< 0.002	< 0.001
Brain	0.036	0.008	0.001	< 0.001	< 0.001	0.057	0.011	< 0.001	< 0.001	< 0.001
Fat (renal)	0.124	0.097	0.008	< 0.002	< 0.001	0.295	0.133	0.004	< 0.001	< 0.001
Gastrointestinal tract	5.372	4.759	1.037	0.139	0.013	5.923	5.126	1.038	0.118	0.017
Gastrointestinal tract contents	11.131	10.284	2.541	0.244	0.025	10.423	10.609	3.443	0.210	0.027
Heart	0.073	0.029	0.009	0.003	0.001	0.098	0.034	0.005	< 0.001	< 0.001
Kidneys	0.208	0.118	0.035	0.013	0.006	0.227	0.161	0.029	0.008	0.004
Liver	1.103	0.512	0.166	0.055	0.025	1.029	0.700	0.088	0.028	0.014
Lungs	0.086	0.036	0.014	0.007	0.002	0.129	0.043	0.009	0.003	< 0.002
Muscle	0.048	0.043	0.004	0.002	< 0.001	0.057	0.021	0.003	< 0.002	< 0.001
Pancreas	0.162	0.050	0.011	0.004	< 0.001	0.317	0.075	0.010	0.002	< 0.001
Plasma	0.079	0.047	0.028	0.014	0.004	0.115	0.063	0.014	0.004	< 0.002
Residual carcass	0.057	0.047	0.026	0.006	< 0.003	0.087	0.040	0.023	0.008	0.005
Spleen	0.060	0.024	0.008	0.003	0.001	0.323	0.027	0.005	< 0.002	< 0.002
Testes/ovaries	0.032	0.017	0.005	0.002	0.001	0.120	< 0.042	0.005	< 0.002	< 0.002
Uterus	—	—	—	—	—	0.073	0.035	0.004	< 0.002	< 0.001
Thymus	0.042	0.015	0.004	< 0.002	< 0.001	0.063	0.021	0.003	< 0.001	< 0.001
Thyroid	0.077	< 0.029	0.027	< 0.009	< 0.008	0.155	< 0.192	0.018	< 0.007	< 0.010
Whole blood	0.058	0.045	0.028	0.014	< 0.005	0.072	0.047	0.013	0.005	0.002

From Shaw (2009e)

Radioactive residues following administration of [pyrazole-5-^{14}C]sedaxane at a dose of 1 mg/kg bw to male rats for 14 days were well distributed in the extensive range of tissues collected (Table 7). Mean concentrations of total radioactivity in each tissue generally increased with each sampling time during the period of dosing and were detectable in most tissues by 24 hours after the seventh dose. Mean tissue concentrations of total radioactivity were at their highest observed levels 24 hours after the 14th dose, with the exception of blood and plasma, for which the highest mean concentrations were observed 24 hours after the 10th dose. Most mean tissue concentrations appeared either to have attained or to be approaching steady-state kinetics by the end of the 14-day dosing period. Following the cessation of dosing, all tissue concentrations declined, with no evidence of any persistence.

Table 5. Distribution of radioactivity in tissues/organs 5, 12, 24, 48 and 96 hours after administration of [pyrazole-5-^{14}C]sedaxane to rats at a dose level of 80 mg/kg bw

	Group mean tissue residues (µg Eq/g or ml)									
	Males					Females				
	5 h	12 h	24 h	48 h	96 h	5 h	12 h	24 h	48 h	96 h
Adrenals	32.39[a]	11.33	1.28	0.50	< 0.27	65.45	21.36	1.66	0.46	< 0.26
Bone mineral	4.23	1.59	0.27	< 0.11	< 0.06	4.71	2.19	0.36	< 0.10	< 0.06
Brain	12.90	1.79	0.13	< 0.05	< 0.03	20.45	3.45	0.11	< 0.03	< 0.01
Fat (renal)	62.74	49.05	3.17	0.17	< 0.04	107.55	73.66	3.25	0.27	< 0.05
Gastrointestinal tract	605.34	358.62	147.20	22.92	1.60	295.64	370.70	142.70	24.55	1.27
Gastrointestinal tract contents	709.60	554.21	261.53	50.41	2.65	626.13	815.33	433.52	55.69	2.40
Heart	18.93	4.09	0.64	0.27	0.14	20.43	6.21	0.63	0.18	< 0.07
Kidneys	35.18	10.43	2.72	1.05	0.59	31.42	13.51	3.34	1.14	0.45
Liver	71.74	40.14	11.88	5.63	2.85	64.56	34.92	11.38	4.08	1.42
Lungs	18.45[b]	4.37	0.85	0.44	0.19	23.24	6.49	1.05	0.35	0.14
Muscle	9.36	3.02	0.42	0.11	0.06	16.99	4.26	0.34	0.11	< 0.03
Pancreas	47.09	11.68	1.01	< 0.22	< 0.07	65.70	15.57	0.70	0.20	< 0.06
Plasma	10.41	3.67	1.02	0.68	< 0.20	15.48	5.59	1.63	0.53	< 0.20
Residual carcass	19.35	7.07	2.67	0.63	0.21	24.69	13.47	5.72	0.71	0.29
Spleen	14.05	7.09	0.55	< 0.24	< 0.19	20.27	6.98	0.79	0.23	0.13
Testes/ovaries	8.16	3.19	0.43	0.14	< 0.07	46.03	19.75	0.92	0.21	< 0.10
Uterus	—	—	—	—	—	24.89	23.95	0.75	0.19	< 0.10
Thymus	28.45	7.34	0.25	< 0.12	< 0.05	17.96	3.85	0.36	< 0.08	< 0.04
Thyroid	19.35[b]	9.67	2.12	1.35	1.05	79.01	7.80	2.52	1.01	< 0.89
Whole blood	7.60	3.53	1.37	0.72	0.38	11.06	4.26	1.54	0.67	0.29

From Shaw (2009e)

[a] 033M removed from the mean due to inconsistency with other values.

[b] 031M removed from the mean due to inconsistency with other values.

Mean tissue levels of radioactivity were highest in the liver and kidney, and these were the only tissues to consistently exceed blood concentrations throughout the study (Table 8). This is consistent with both biliary and urinary elimination of [pyrazole-5-^{14}C]sedaxane and its metabolites. After the liver and kidney, the thyroid had the next highest mean concentration of radioactivity during the study, followed by the adrenals and spleen. Although thyroid appeared to show some accumulation of radioactivity during dosing, the mean concentration in the thyroid was no longer at a reliably measurable level by 28 days after the 14th dose. Residues in all other tissues were generally below blood and plasma concentrations throughout the course of the study. By the final sampling time (42 days after the 14th dose), mean concentrations of radioactivity were measurable only in the liver, kidney and spleen. The terminal half-life for tissue depletion was variable, with the shortest estimate in the plasma and longest in the spleen, 2.3 days and 33.0 days, respectively (Shaw, 2009f).

Table 6. Elimination of radioactivity from rat tissues/organs after a single administration of [pyrazole-5-^{14}C]sedaxane to rats at a dose level of 1 or 80 mg/kg bw

Tissue	Elimination half-life (h)[a]			
	1 mg/kg bw		80 mg/kg bw	
	Males	Females	Males	Females
Adrenals	11.58	10.62	40.37	7.00
Bone mineral	13.10	16.79	10.92	10.21
Brain	4.59	3.97	2.90	2.51
Fat (renal)	8.34	3.53	4.56	4.68
Heart	27.23	9.11	33.75	27.70
Kidneys	30.62	27.90	34.77	26.02
Liver	27.87	28.52	36.45	24.83
Lungs	25.51	36.11	34.15	26.49
Muscle	42.46	13.87	28.03	21.85
Ovaries	NA	8.42	NA	25.63
Pancreas	21.30	7.39	20.63	25.22
Plasma	25.84	27.53	32.33	27.67
Spleen	25.19	11.05	59.22	30.47
Testes	30.88	NA	29.82	NA
Thymus	14.02	5.01	6.32	7.47
Thyroid	48.38	NC	75.88	47.69
Uterus	NA	10.23	NA	26.70
Whole blood	29.49	31.22	40.10	31.01

From Shaw (2009e)
NA, not applicable; NC, not calculated
[a] Each value is a mean of three rats.

Table 7. Distribution of radioactivity in tissues/organs 24 hours after days 3, 7, 10 and 14 of a repeated administration of [pyrazole-5-^{14}C]sedaxane to male rats at a dose of 1 mg/kg bw

	Group mean tissue residues (µg Eq/g or ml)			
	Day 3	Day 7	Day 10	Day 14
Adrenals	< 0.032	< 0.033	0.047	0.099
Bone mineral	< 0.012	0.010	0.012	0.032
Brain	< 0.003	< 0.005	< 0.003	0.013
Fat (renal)	< 0.013	0.013	0.008	0.023
Gastrointestinal tract	1.609	2.191	1.414	2.639
Gastrointestinal tract contents	4.553	6.120	5.010	5.496
Heart	0.013	0.020	0.024	0.026
Kidney	0.066	0.119	0.094	0.194
Liver	0.287	0.460	0.416	0.507
Lungs	0.023	0.037	0.041	0.044
Pancreas	0.018	0.032	0.017	0.022
Plasma	0.037	0.060	0.088	0.066
Residual carcass	0.034	0.044	0.068	0.083

Table 7 (continued)

	Group mean tissue residues (µg Eq/g or ml)			
	Day 3	Day 7	Day 10	Day 14
Spleen	0.013	0.019	0.022	0.027
Testes	0.008	0.013	0.013	0.013
Thymus	< 0.008	0.010	0.011	0.013
Thyroid	< 0.050	< 0.087	0.140	0.189
Whole blood	0.037	0.062	0.079	0.070

From Shaw (2009f)

Table 8. Distribution of radioactivity in tissues/organs up to 42 days after day 14 of repeated administration of [pyrazole-5-^{14}C]sedaxane to male rats at a dose of 1 mg/kg bw

	Group mean tissue residues (µg Eq/g or ml)						
	Day 3	Day 7	Day 10	Day 14	Day 21	Day 28	Day 42
Adrenals	< 0.022	0.028	< 0.034	< 0.010	< 0.026	< 0.005	< 0.009
Bone mineral	< 0.007	< 0.005	< 0.008	< 0.002	< 0.006	< 0.002	< 0.002
Brain	< 0.002	< 0.002	< 0.005	< 0.001	< 0.001	< 0.001	< 0.001
Fat (renal)	< 0.003	< 0.002	< 0.014	< 0.002	< 0.002	< 0.001	< 0.001
Gastrointestinal tract	0.192	0.025	0.007	0.004	0.004	< 0.002	< 0.002
Gastrointestinal tract contents	0.355	0.049	0.011	0.005	0.004	< 0.002	< 0.001
Heart	0.009	0.006	0.026	< 0.003	< 0.003	< 0.001	< 0.001
Kidneys	0.056	0.050	0.039	0.024	0.025	0.014	0.008
Liver	0.177	0.146	0.087	0.048	0.037	0.027	0.009
Lungs	0.017	0.010	0.007	< 0.004	< 0.003	< 0.002	< 0.002
Muscle	0.005	< 0.004	0.004	< 0.001	< 0.002	< 0.001	< 0.001
Pancreas	< 0.007	< 0.004	< 0.006	< 0.001	< 0.002	< 0.001	< 0.001
Plasma	0.034	0.011	< 0.002	< 0.001	< 0.001	< 0.001	< 0.001
Residual carcass	0.028	0.022	0.022	0.010	0.010	0.006	< 0.005
Spleen	0.014	0.012	0.015	0.008	0.008	0.007	0.005
Testes	0.006	0.003	< 0.003	< 0.001	< 0.001	0.006	0.003
Thymus	< 0.005	< 0.003	< 0.006	< 0.001	< 0.002	< 0.001	< 0.001
Thyroid	0.082	< 0.066	0.071	0.048	0.028	< 0.034	< 0.024
Whole blood	0.035	0.022	0.014	0.007	0.006	< 0.002	< 0.002

From Shaw (2009f)

(d) Excretion

A single oral dose of [pyrazole-5-^{14}C]sedaxane (lot nos CL-LXII-15 for low dose, CL-LXII-14 for high dose; purity 99.2% and 97.0% for low and high doses, respectively; ratios of isomers 84.6% *trans* to 15.4% *cis* for low dose and 85.9% *trans* to 14.1% *cis* for high dose) was administered by gavage to eight male and eight female rats (Han Wistar) at a dose of either 1 or 80 mg/kg bw. The excretion of radioactivity was measured over 7 days. After this period, the rats were killed, and residual radioactivity was measured in blood and plasma, selected tissues and the remaining carcasses.

The major route of elimination of a single oral dose of [pyrazole-5-^{14}C]sedaxane at 1 mg/kg bw was via the faeces in both males and females, with respective means of 88.4% and 79.4% of the administered radioactivity recovered by this route over 7 days after dosing. Urinary excretion accounted for means of 11.8% and 19.6% of the administered dose in males and females, respectively, by the end of the sampling period (Table 9).

Table 9. Recovery of radioactivity in excreta and tissues after administration of a single oral dose of [pyrazole-^{14}C]sedaxane to rats

		Group mean excretion (% of radioactive dose recovered)[a]			
		1 mg/kg bw		80 mg/kg bw	
		Males (n = 4)	Females (n = 4)	Males (n = 4)	Females (n = 4)
Urine	0–6 h	3.0	4.0	2.0	1.7
	6–12 h	3.7	6.5	2.0	3.0
	12–24 h	3.0	6.2	3.9	6.8
	24–48 h	1.6	2.4	2.7	4.3
	48–72 h	0.3	0.4	0.9	1.4
	72–96 h	0.1	0.1	0.3	0.2
	96–120 h	< 0.1	0.1	0.1	< 0.1
	120–144 h	< 0.1	< 0.1	< 0.1	< 0.1
	144–168 h	< 0.1	< 0.1	< 0.1	< 0.1
	Subtotal	11.8	19.6	11.9	17.6
Faeces	0–24 h	68.3	57.8	41.0	36.3
	24–48 h	16.2	19.4	28.1	28.7
	48–72 h	2.9	1.8	11.1	8.8
	72–96 h	0.7	0.3	2.3	0.9
	96–120 h	0.3	0.1	0.4	0.2
	120–144 h	0.1	< 0.1	0.1	0.1
	144–168 h	< 0.1	< 0.1	0.1	< 0.1
	Subtotal	88.4	79.4	83.1	74.9
Cage wash		1.7	5.8	2.0	3.7
Gastrointestinal tract + contents		< 0.1	< 0.1	< 0.1	< 0.1
Tissues + carcass		0.2	0.1	0.3	0.1
Total recovery		102.1	104.9	97.2	96.3

From Shaw (2009a)

[a] Total and subtotal values may not add up exactly due to rounding of values.

The major route of elimination of a single oral dose of [phenyl-U-^{14}C]sedaxane at 80 mg/kg bw was similarly via the faeces in both males and females, with respective means of 83.1% and

74.9% of the administered radioactivity recovered by this route over 7 days post-dosing. Urinary excretion accounted for means of 11.9% and 17.6% of the administered dose in males and females, respectively, by the end of the sampling period (Table 9).

Excretion was rapid, with essentially all administered radioactivity being eliminated in the first 72 hours after dosing (means of approximately 100.4% and 103.5% in males and females, respectively, for the low dose and approximately 93.4% and 94.6% in males and females, respectively, for the high dose). The routes and rates of excretion were similar in both sexes, although urinary excretion of absorbed components was slightly higher in females. Seven days after administration of the low and high doses, there was no significant radioactivity remaining in the carcass or gastrointestinal tract, indicating that excretion was essentially complete by 7 days after the single administered dose.

A single oral dose of [pyrazole-^{14}C]sedaxane at 1 or 80 mg/kg bw was rapidly and extensively eliminated, irrespective of dose level or sex. At both dose levels, the major route of elimination was via the faeces, and faecal elimination was slightly higher in males than in females. Accordingly, urinary elimination was slightly higher in females. At both dose levels, residues of radioactivity were very low in blood and tissues by 7 days after dosing and were reliably detected in both sexes only in the liver and kidney. Tissue distribution was generally similar in both sexes at both dose levels, These very low tissue residues were consistent with the extensive excretion of the administered dose (Shaw, 2009a).

1.2 Biotransformation

The biotransformation of sedaxane was investigated using [pyrazole-5-^{14}C]sedaxane and [phenyl-U-^{14}C]sedaxane (with a *trans*:*cis* isomer ratio of approximately 6:1) administered in 14 consecutive daily oral doses (low dose level) to male rats and single high and single low oral doses administered to both intact and bile duct–cannulated male and female rats that had been used in previous studies (Shaw, 2009a,b,c,d,f). All metabolites accounting for greater than 5% of the administered dose were identified, and the majority of those accounting for less than 5% were tentatively identified; proposed chemical structures were assigned. Metabolites were identified by radio–high-performance liquid chromatography with mass spectrometry using a combination of comparative chromatography with authentic reference standards, accurate mass measurement and MS^n fragmentation. Minor metabolites were tentatively identified and assigned a proposed chemical structure based on mass spectrometric data. Selected bile and urine samples were subjected to enzyme hydrolysis using a mixture of β-glucuronidase and sulfatase enzymes to assist in the identification of conjugated metabolites.

Sedaxane was extensively metabolized, giving rise to at least 20 types of metabolite (e.g. hydroxy, demethylated hydroxy, glucuronide conjugate, sulfate conjugate), with the potential for multiple isomers within most types. No significant differences in the nature of the metabolites identified were observed in low- and high-dose male and female rats, although some quantitative variation was observed. There was little evidence of significant cleavage of sedaxane into the pyrazole and phenyl moieties, similar metabolic profiles being observed in samples from rats receiving pyrazole- or phenyl-labelled [^{14}C]sedaxane. Small amounts (< 1%) of CSCC210616 (pyrazole amide metabolite) were detected in bile samples. Phenolic and hydroxy metabolites of sedaxane and desmethyl sedaxane were conjugated with glucuronic acid, sulfate and glutathione. Identification of the aglycones following enzymatic hydrolysis using a mixture of β-glucuronidase and sulfatase enzymes confirmed the structural assignments of these conjugated metabolites. Generally, when identification allowed, the metabolites derived from the *trans* isomer of sedaxane were the major components of the metabolic profile. Measurement of the isomers of the remaining sedaxane in samples from rats receiving the low dose of [pyrazole-5-^{14}C]sedaxane showed an approximately similar ratio of *trans* to *cis* isomers as that of the sedaxane administered (ranging from 1.49:1 in plasma to 7.96:1 in faeces from intact rats). In plasma, urine and bile samples from rats receiving the high dose, a much greater proportion of the *trans* isomer (ranging from 12.18:1 in urine from bile duct–cannulated rats to 45.96:1 in plasma from intact rats) was observed.

The biotransformation of sedaxane was postulated to proceed by:

- *N*-demethylation;
- hydroxylation of sedaxane to give the *para*-phenols CSCD658906 and CSCD659090 and the cyclopropyl alcohol CSCD659089; these metabolites were excreted primarily in the bile as glucuronide conjugates;
- hydroxylation of desmethyl sedaxane to give the desmethyl *para*-phenols CSCD659087 and CSCD668404 and the desmethyl cyclopropyl alcohol CSCD659088; these metabolites were excreted primarily in the bile as glucuronide conjugates;
- opening of the terminal cyclopropyl moiety followed by oxidation of the sedaxane and desmethyl sedaxane to give β-hydroxycarbonyl sedaxane (the *trans* isomer CSCD668403 was identified) and β-hydroxycarbonyl desmethyl sedaxane metabolites;
- further hydroxylation and oxidation of hydroxyl and desmethyl hydroxyl metabolites;
- further hydroxylation and oxidation of β-hydroxycarbonyl sedaxane and desmethyl β-hydroxycarbonyl sedaxane metabolites;
- glucuronic acid conjugation together with minor amounts of sulfate conjugation of hydroxylated metabolites;
- glutathione conjugation.

The metabolite profiles in excreta of intact or bile duct–cannulated rats following single or repeated oral doses of [pyrazole-5-^{14}C]sedaxane and [phenyl-U-^{14}C]sedaxane at low and high doses are shown in Tables 10–16.

Table 10. Metabolite profile in excreta of rats following a single oral dose of [pyrazole-5-^{14}C]sedaxane at the high dose of 80 mg/kg bw

Compound	% of administered dose (number of isomers)					
	Males			Females		
	Urine (0–96 h)	Faeces (0–96 h)	Total excreta	Urine (0–96 h)	Faeces (0–96 h)	Total excreta
Sedaxane	—	4.25	4.25	—	1.68	1.68
CSCD659087	1.68	15.38	17.06	2.85	22.41	25.26
CSCD668404	0.24	3.04	3.28	0.27	6.99	7.26
CSCD659088	—	2.08	2.08	—	0.96	0.96
CSCD658906	1.70	16.83	18.53	2.02	16.65	18.67
CSCD658089	—	9.77	9.77	—	1.22	1.22
CSCD659090	—	4.27[a]	4.27	0.26	3.37[a]	3.63
CSCD668403	0.25	—	0.25	0.25	—	0.25
β-Hydroxycarbonyl cysteine conjugate	—	0.83	0.83	—	0.75	0.75
Desmethyl hydroxy	—	2.95[a]	2.95	—	2.43[a]	2.43
Desmethyl dihydroxy	—	0.99 (1)	0.99	—	1.84 (3)	1.84
Desmethyl β-hydroxycarbonyl	1.22 (1)	—	1.22	4.78 (1)[b]	—	4.78
Dihydroxy	0.20 (1)	5.03 (3)	5.23	0.23 (1)	2.78 (2)	3.01

Table 10 (continued)

Compound	% of administered dose (number of isomers)					
	Males			Females		
	Urine (0–96 h)	Faeces (0–96 h)	Total excreta	Urine (0–96 h)	Faeces (0–96 h)	Total excreta
Dihydroxy/β-hydroxycarbonyl[c]	0.40 (1)[c]	—	0.40	0.17 (1)[c]	—	0.17
Carboxylic acid	0.73 (1)	—	0.73	—	—	
Hydroxy β-hydroxycarbonyl	2.02 (5)	2.36 (1)	4.38	0.19 (1)	1.54 (1)	1.73
Desmethyl hydroxy sulfate conjugate	1.32 (1)	—	1.32	—	—	—
Desmethyl glucuronide	—	(2)[d]	—	—	(2)[d]	—
Desmethyl hydroxy glucuronide	—	—	—	0.48 (2)[e]	—	0.48
Hydroxy glucuronide	—	2.02 (1)	2.02	3.08 (1)	0.73	3.81
Post-extraction solids	NA	10.00	10.00	NA	8.20	8.20
Total identified	9.76	69.80	79.56	14.58	63.35	77.93
Total unidentified	2.83	1.20	4.03[f]	3.63	2.55	6.18[g]
Total accounted for	12.59	81.00	93.59	18.21	74.10	92.31
Losses/gains[h]	−0.99	0.80	−0.19	−1.11	0.70	−0.41
Total	11.60	81.80	93.40	17.10	74.80	91.90

From Green (2009)
NA, not applicable
[a] Includes an unresolved desmethyl glucuronide metabolite.
[b] Includes an unresolved desmethyl hydroxy glucuronide (phenolic) metabolite.
[c] The empirical formula for these structures is the same; therefore, this metabolite could not be identified based on accurate mass measurement. With the exception of urine obtained from female rats administered (^{14}C-pyrazole)-labelled sedaxane (80 mg/kg bw), in which this component was identified as a β-hydroxycarbonyl, fragmentation data were not available to define the identification.
[d] Two desmethyl glucuronide metabolites were detected and unresolved from CSCD659090 and desmethyl hydroxysedaxane.
[e] Two desmethyl hydroxy glucuronide metabolites were detected, one unresolved from a desmethyl β-hydroxycarbonyl metabolite.
[f] Ten components, none greater than 0.68% of the administered dose.
[g] Eleven components, none greater than 0.89% of the administered dose.
[h] Losses/gains on fractionation incorporate procedural losses. This is calculated from the sum of the per cent administered dose in each component subtracted from the per cent administered dose in sample.

Table 11. Metabolite profile in excreta of rats following a single oral dose of [pyrazole-5-^{14}C]sedaxane at the low dose of 1 mg/kg bw

Compound	% of administered dose (number of isomers)					
	Males			Females		
	Urine (0–96 h)	Faeces (0–96 h)	Total excreta	Urine (0–96 h)	Faeces (0–96 h)	Total excreta
Sedaxane	—	1.71	1.71	—	1.56	1.56
CSCD659087	2.58	26.86	29.44	9.33	29.16	38.49
CSCD668404	0.35	5.08	5.43	1.55	9.32	10.87
CSCD659088	—	1.32	1.32	—	1.47	1.47
CSCD658906	1.46	10.05	11.51	2.34	11.74	14.08

Compound	% of administered dose (number of isomers)					
	Males			Females		
	Urine (0–96 h)	Faeces (0–96 h)	Total excreta	Urine (0–96 h)	Faeces (0–96 h)	Total excreta
CSCD659089	—	5.05	5.05	—	—	—
CSCD659090	—	2.29[a]	2.29	0.28	1.98[a]	2.26
Desmethyl hydroxy	—	1.26 (1)[a]	1.26	—	2.99 (1)[a]	2.99
Desmethyl dihydroxy	—	2.17 (1)	2.17	—	1.63 (1)	1.63
Desmethyl β-hydroxycarbonyl	2.11 (1)	—	2.11	2.71 (1)[b]	—	2.71
Dihydroxy	0.22 (1)	5.17 (3)	5.39	—	1.56 (1)	1.56
Dihydroxy/β-hydroxycarbonyl[c]	0.30 (1)[c]	—	0.30	—	—	—
Carboxylic acid	0.56 (1)	—	0.56	—	—	—
Hydroxy β-hydroxycarbonyl	0.70 (3)	0.88 (1)	1.58	—	0.80 (1)	0.80
Desmethyl hydroxy sulfate conjugate	0.42 (1)	—	0.42	—	—	—
Desmethyl glucuronide	—	(2)[d]	—	—	(2)[d]	—
Desmethyl hydroxy glucuronide	—	—	—	0.36 (2)[e]	—	0.36
Hydroxy glucuronide	—	1.21 (1)	1.21	0.96 (1)	2.10 (1)	3.06
Post-extraction solids	NA	13.60	13.60	NA	11.00	11.00
Total identified	8.70	63.05	71.75	17.53	64.31	81.84
Total unidentified	3.01	10.66	13.67[f]	1.87	3.30	5.17[g]
Total accounted for	11.71	87.31	99.02	19.40	78.61	98.01
Losses/gains[h]	−0.01	1.09	1.08	−0.20	0.79	0.59
Total	11.70	88.40	100.1	19.20	79.40	98.60

From Green (2009)

NA, not applicable

[a] Includes an unresolved desmethyl glucuronide metabolite.

[b] Includes an unresolved desmethyl hydroxy glucuronide (phenolic) metabolite.

[c] The empirical formula for these structures is the same; therefore, this metabolite could not be identified based on accurate mass measurement. With the exception of urine obtained from female rats administered (^{14}C-pyrazole)-labelled sedaxane (80 mg/kg bw), in which this component was identified as a β-hydroxycarbonyl, fragmentation data were not available to define the identification.

[d] Two desmethyl glucuronide metabolites were unresolved from CSCD659090 and desmethyl hydroxy sedaxane.

[e] Two desmethyl hydroxy glucuronide metabolites were detected, one unresolved from a desmethyl β-hydroxycarbonyl metabolite.

[f] Fifteen components, none greater than 1.98% of the administered dose.

[g] Nine components, none greater than 1.60% of the administered dose.

[h] Losses/gains on fractionation incorporate procedural losses. This is calculated from the sum of the per cent administered dose in each component subtracted from the per cent administered dose in sample.

Table 12. Metabolite profile in excreta of male rats following a repeated oral dosing of [pyrazole-5-^{14}C]sedaxane at the low dose of 1 mg/kg bw

Compound	% of administered dose (number of isomers)					
	0–24 h			312–336 h		
	Urine	Faeces	Total excreta	Urine	Faeces	Total excreta
Sedaxane	—	3.86	3.86	—	—	—
CSCD659087	3.56	21.70	25.26	1.03	35.14	36.17
CSCD668404	0.40	5.26	5.66	0.25	7.46	7.71
CSCD659088	—	—	—	—	—	—
CSCD658906	1.52	7.32	8.84	0.59	11.53	12.12
CSCD659089	—	5.33[a]	5.33	—	2.92	2.92
CSCS659090	—	(1)[b]	—	—	2.03	2.03
Desmethyl β-hydroxycarbonyl	2.96 (1)[c]	—	2.96	5.76 (1)[c]	—	5.76
Dihydroxy/β-hydroxycarbonyl[d]	0.95 (1)[d]	—	0.95	0.95 (1)[d]	—	0.95
Desmethyl hydroxy sulfate conjugate	—	—	—	0.48 (1)	—	0.48
Desmethyl glucuronide	—	(1)[b]	—	—	—	—
Desmethyl hydroxy glucuronide	1.00 (2)[e]	—	1.00	0.64 (2)[e]	—	0.64
Hydroxy glucuronide	1.58 (1)	—	1.58	4.29 (1)	—	4.29
Post-extraction solids	NA	12.10	12.10	NA	8.70	8.70
Total identified	11.97	43.47	55.44	13.99	59.08	72.43
Total unidentified	1.03	4.73	5.76[f]	1.31	3.33	4.64[g]
Total accounted for	13.00	60.30	73.30	15.30	71.11	85.77
Losses/gains[h]	0.00	0.60	0.60	0.60	0.89	2.13
Total	13.00	60.90	73.90	15.90	72.00	87.90

From Green (2009)

NA, not applicable

[a] Includes unresolved components CSCD659090 and a desmethyl glucuronide metabolite.

[b] Component detected within a region of unresolved components, including CSCD659089, CSCD659090 and a desmethyl glucuronide metabolite.

[c] Includes an unresolved desmethyl hydroxy glucuronide metabolite.

[d] The empirical formula for these structures is the same; therefore, this metabolite could not be identified based on accurate mass measurement.

[e] Two desmethyl hydroxy glucuronide metabolites were detected; one was unresolved from a desmethyl β-hydroxycarbonyl metabolite.

[f] Four components, none greater than 4.73% of the administered dose.

[g] Three components, none greater than 3.33% of the administered dose.

[h] Losses/gains on fractionation incorporate procedural losses. This is calculated from the sum of the per cent administered dose in each component subtracted from the per cent administered dose in sample.

Table 13. Metabolite profile in excreta of bile duct–cannulated rats following a single oral dose of [pyrazole-5-^{14}C]sedaxane at the high dose of 80 mg/kg bw

Compound	% of administered dose (number of isomers)									
	Males					Females				
	Urine (0–48 h)	Faeces (0–48 h)	Bile (0.5–48 h)		Total excreta[a]	Urine (0–48 h)	Faeces (0–48 h)	Bile (0.5–48 h)		Total excreta[a]
			U	H				U	H	
Sedaxane	0.05	6.99[b]	1.40	0.59	8.44	—	2.69[b]	0.98	—	3.67
CSCC210616	—	—	0.96	—	0.96	—	—	0.52	—	0.52
CSCD659087	0.22	—	2.34	14.70	2.56	1.89	—	1.67	20.10	3.56
CSCD668404	—	—	1.01[c]	2.15	1.01	0.34	—	0.85[c]	5.09	1.19
CSCD659088	—	—	0.59	1.60	0.59	—	—	—	1.37	—
CSCD658906	0.23	—	5.30	30.87	5.53	1.84	—	3.15	34.89	4.99
CSCD659089	—	—	—	9.85	—	—	—	—	5.09	—
CSCS659090	0.70	—	2.19[d]	7.53	2.89	—	—	2.18[d]	8.20	2.18
CSCD668403	—	—	—	—	—	0.18	—	—	—	0.18
β-Hydroxycarbonyl cysteine conjugate	—	—	—	0.52 (1)	—	—	—	—	—	—
Desmethyl sedaxane	—	—	—	—	—	—	0.41 (1)	—	—	0.41
Hydroxy	—	—	—	0.96 (1)	—	—	—	—	—	—
Desmethyl β-hydroxycarbonyl	0.73 (1)[e]	—	—	—	0.73	1.29 (1)[e]	—	—	—	1.29
Dihydroxy/β-hydroxycarbonyl[f]	0.16 (1)[f]	—	—	—	0.16	0.33 (1)[g]	—	—	—	0.33
β-Hydroxycarbonyl	—	—	—	—	—	0.57 (1)	—	—	—	0.57
Dihydroxy	—	—	—	0.74 (1)	—	—	—	—	—	—
Carboxylic acid	0.49 (1)	—	—	—	0.49	0.34 (1)	—	—	—	0.34
Hydroxy β-hydroxycarbonyl	0.76 (4)	—	—	—	0.76	0.37 (1)	—	—	—	0.37
Hydroxy sulfate conjugate	0.28 (1)	—	—	—	0.28	0.16 (1)	—	—	—	0.16
Hydroxy cysteine conjugate	—	—	—	0.15 (1)	—	—	—	—	—	—
Desmethyl glucuronide	—	—	1.18 (2)[h]	2.59 (1)	1.18	0.90 (2)	—	1.10 (2)[h]	3.25 (1)	2.00
Desmethyl hydroxy glucuronide	0.09 (2)[i]	—	16.87 (5)[j]	—	16.96	0.20 (2)[i]	—	23.82 (4)[k]	—	24.02
Hydroxy glucuronide	0.32 (1)	—	38.91 (4)[l]	—	40.25	0.71(2)[m]	—	43.40 (4)[l]	—	44.11

Compound	% of administered dose (number of isomers)									
	Males					Females				
	Urine (0–48 h)	Faeces (0–48 h)	Bile (0.5–48 h)		Total excreta[a]	Urine (0–48 h)	Faeces (0–48 h)	Bile (0.5–48 h)		Total excreta[a]
			U	H				U	H	
Dihydroxy glucuronide	—	—	1.11 (1)	—	1.11	—	—	—	—	—
Hydroxy glutathione conjugate	—	—	(1)[n]	—	—	—	—	0.32 (1)[n]	—	0.32
Dihydroxy glutathione	—	—	0.61 (1)	—	0.61	—	—	—	—	—
Post-extraction solids	NA	0.20	NA	NA	0.20	NA	0.20	NA	NA	0.20
Total identified	4.03	6.99	72.47	72.25	83.49	9.12	3.10	77.99	77.99	90.21
Total unidentified	1.67	0.00	1.84	2.05	3.51[o]	0.68	0.00	0.00	0.00	0.68[p]
Total accounted for	5.70	7.19	74.31	74.30	87.20	9.80	3.30	77.99	77.99	91.09
Losses/gains[q]	0.20	0.03	7.49	7.50	7.72	0.40	−0.06	3.21	3.21	3.55
Total	5.90	7.22	81.80	81.80	94.92	10.20	3.24	81.20	81.20	94.64

From Green (2009)

H, hydrolysed; NA, not applicable; U, unhydrolysed

[a] Sum of urine, faeces and unhydrolysed bile.

[b] Postulated to include the *trans* and *cis* isomers comprising sedaxane.

[c] Includes an unresolved hydroxy glucuronide (phenolic) metabolite.

[d] Includes an unresolved desmethyl glucuronide metabolite.

[e] Includes an unresolved desmethyl hydroxy glucuronide metabolite.

[f] The empirical formula for these structures is the same; therefore, this metabolite could not be identified based on accurate mass.

[g] Includes an unresolved hydroxy glucuronide metabolite.

[h] Two desmethyl glucuronide metabolites were detected, one unresolved from CSCD659090.

[i] Two desmethyl hydroxy glucuronide metabolites were detected, one unresolved from a desmethyl β-hydroxycarbonyl metabolite.

[j] Includes two hydroxy glutathione metabolites unresolved from a desmethyl hydroxy glucuronide metabolite.

[k] Includes a hydroxy glutathione metabolite unresolved from an isomer of a desmethyl hydroxy glucuronide metabolite.

[l] Four hydroxy glucuronide metabolites were detected, one unresolved from CSCD668404.

[m] Two isomers of hydroxy glucuronide metabolites were detected, one unresolved from a dihydroxy/β-hydroxycarbonyl metabolite.

[n] One hydroxy glutathione metabolite was unresolved from a desmethyl hydroxy glucuronide metabolite.

[o] Eleven components, none greater than 1.04% of the administered dose.

[p] Three components, none greater than 0.36% of the administered dose.

[q] Losses/gains on fractionation incorporate procedural losses. This is calculated from the sum of the per cent administered dose in each component subtracted from the per cent administered dose in sample.

Table 14. Metabolite profile in excreta of bile duct–cannulated rats following a single oral dose of [pyrazole-5-^{14}C]sedaxane at the low dose of 1 mg/kg bw

Compound	% of administered dose (number of isomers)									
	Males					Females				
	Urine (0–48 h)	Faeces (0–48 h)	Bile (0.5–48 h)		Total excreta[a]	Urine (0–48 h)	Faeces (0–48 h)	Bile (0.5–48 h)		Total excreta[a]
			U	H				U	H	
Sedaxane	—	6.41	0.34	0.72	6.75	—	4.49	0.73	—	5.22
CSCC210616	—	—	0.16	—	0.16	—	—	0.84	—	0.84
CSCD659087	1.04	—	3.46	25.41	4.50	2.36	—	3.05	28.80	5.41
CSCD668404	—	—	0.96[b]	5.13	0.96	0.50	—	1.17[b]	6.59	1.67
CSCD659088	—	—	—	1.32	—	—	—	—	1.52	—
CSCD658906	0.86	—	2.30	22.46	3.16	0.62	—	3.06	25.03	3.68
CSCD659089	—	—	—	4.99	—	—	—	—	2.33	—
CSCS659090	—	—	1.06[c]	5.63	1.06	—	—	0.57[c]	5.99	0.57
Hydroxy	—	—	—	0.57	—	—	—	—	—	—
Desmethyl β-hydroxycarbonyl	0.87[d]	—	—	—	0.87	1.66[d]	—	—	—	1.66
Dihydroxy/β-hydroxycarbonyl[e]	0.39[e]	—	—	—	0.39	—	—	—	—	—
β-Hydroxycarbonyl	—	—	—	—	—	0.22 (1)	—	—	—	0.22
Dihydroxy	—	—	—	0.60	—	—	—	—	—	—
Carboxylic acid	0.36	—	—	—	0.36	0.23	—	—	—	0.23
Hydroxy β-hydroxycarbonyl	0.42	—	—	—	0.42	—	—	—	—	—
Hydroxy cysteine conjugate	—	—	—	2.77	—	—	—	—	—	—
Desmethyl glucuronide	—	—	0.51 (2)[f]	3.07	0.51	—	—	1.20 (2)[f]	3.03	1.20
Desmethyl hydroxy glucuronide	(1)[g]	—	31.68 (4)[h]	—	31.68	(1)[g]	—	33.56 (4)	—	33.56
Hydroxy glucuronide	0.47	—	31.81 (4)[i]	—	32.28	0.47 (1)	—	30.20 (4)[i]	—	30.67
Dihydroxy glucuronide	—	—	0.85 (1)	—	0.85	—	—	1.25 (1)	—	1.25
Hydroxy glutathione	—	—	(1)[j]	—	—	—	—	—	—	—

Compound	% of administered dose (number of isomers)									
	Males					Females				
	Urine (0–48 h)	Faeces (0–48 h)	Bile (0.5–48 h)		Total excreta[a]	Urine (0–48 h)	Faeces (0–48 h)	Bile (0.5–48 h)		Total excreta[a]
			U	H				U	H	
conjugate										
Dihydroxy glutathione	—	—	0.32 (1)	—	0.32	—	—	—	—	—
Post-extraction solids	NA	0.30	NA	NA	0.30	NA	0.20	NA	NA	0.20
Total identified	4.41	6.41	73.45	72.67	84.27	6.06	4.49	75.63	73.29	86.18
Total unidentified	2.10	0.00	4.97	5.75	7.07[k]	0.34	0.00	4.19	6.52	4.53[l]
Total accounted for	6.51	6.71	78.42	78.42	91.64	6.40	4.69	79.82	79.81	90.91
Losses/gains[m]	0.19	0.01	0.58	0.58	1.28	0.50	0.04	−0.42	−0.41	0.12
Total	6.70	6.70	79.00	79.00	92.92	6.90	4.73	79.40	79.40	91.03

From Green (2009)

H, hydrolysed; NA, not applicable; U, unhydrolysed

[a] Sum of urine, faeces and unhydrolysed bile.

[b] Includes an unresolved hydroxy glucuronide (phenolic) metabolite.

[c] Includes an unresolved desmethyl glucuronide metabolite.

[d] Includes an unresolved desmethyl hydroxy glucuronide metabolite.

[e] The empirical formula for these structures is the same; therefore, this metabolite could not be identified based on accurate mass.

[f] Two desmethyl glucuronide metabolites were detected, one unresolved from CSCD659090.

[g] A desmethyl hydroxy glucuronide metabolite was unresolved from a desmethyl β-hydroxycarbonyl metabolite.

[h] Includes a hydroxy glutathione metabolite unresolved from a desmethyl hydroxy glucuronide metabolite.

[i] Four hydroxy glucuronide metabolites were detected, one unresolved from CSCD668404.

[j] A hydroxy glutathione metabolite was unresolved from a desmethyl hydroxy glucuronide metabolite.

[k] Eleven components, none greater than 1.96% of the administered dose.

[l] Four components, none greater than 2.59% of the administered dose.

[m] Losses/gains on fractionation incorporate procedural losses. This is calculated from the sum of the per cent administered dose in each component subtracted from the per cent administered dose in sample.

Table 15. Metabolite profile in excreta of bile duct–cannulated rats following a single oral dose of [phenyl-U-^{14}C]sedaxane at the high dose of 80 mg/kg bw

Compound	% of administered dose (number of isomers)							
	Males				Females			
	Urine (0–48 h)	Faeces (0–48 h)	Bile (0.5–48 h)	Total excreta	Urine (0–48 h)	Faeces (0–48 h)	Bile (0.5–48 h)	Total excreta
Sedaxane	—	3.69	0.87	4.56	—	9.60	—	9.60
CSCD659087	0.31	—	0.75	1.06	1.30	—	0.57	1.87
CSCD668404	—	—	1.35[a]	1.35	0.21	0.20	0.47[a]	0.88
CSCD658906	0.29	0.27	1.55	2.11	0.68	0.31	0.69	1.68
CSCD659090	—	—	1.88[b]	1.88	—	—	2.42[b]	2.42
Desmethyl sedaxane	—	—	—	—	—	0.19 (1)	—	0.19
Desmethyl β-hydroxycarbonyl	0.44 (1)	0.23 (1)	—	0.67	0.96 (1)[c]	—	—	0.96
Dihydroxy/β-hydroxycarbonyl[d]	0.67 (1)[d]	—	—	0.67	0.27 (1)[d]	—	—	0.27
Carboxylic acid	0.56 (1)	—	—	0.56	0.21 (1)	—	—	0.21
Hydroxy β-hydroxycarbonyl	0.50 (4)	—	—	0.50	—	—	—	—
Desmethyl hydroxy sulfate conjugate	0.47 (1)	—	—	0.47	—	—	—	—
Hydroxy sulfate conjugate	0.16 (1)	—	—	0.16	—	—	—	—
Desmethyl glucuronide	—	—	2.11 (2)[e]	2.11	0.38 (2)	—	0.86 (2)[e]	1.24
Desmethyl hydroxy glucuronide	0.58 (2)	—	32.81 (6)[f]	33.39	(1)[g]	—	39.44 (4)[f]	39.44
Hydroxy glucuronide	0.63 (2)	—	48.78 (4)[h]	49.41	0.40 (1)	—	40.71 (4)[h]	41.11
Hydroxy glutathione conjugate	—	—	(1)[i]	—	—	—	(1)[i]	—
Post-extraction solids	NA	0.20	NA	0.20	NA	0.20	NA	0.20
Total identified	4.61	4.19	90.10	98.90	4.41	10.30	85.16	99.87
Total unidentified	1.09	0.00	0.00	1.09[j]	0.50	0.00	0.34	0.84[k]
Total accounted for	5.70	4.39	90.10	100.19	4.91	10.5	85.50	100.91
Losses/gains[l]	1.00	0.01	−4.80	−3.79	0.39	0.1	−4.50	−4.01
Total	6.70	4.40	85.30	96.40	5.30	10.6	81.00	96.90

From Green (2009)
NA, not applicable
[a] Includes an unresolved hydroxy glucuronide (phenolic) metabolite.
[b] Includes an unresolved desmethyl glucuronide metabolite.
[c] Includes an unresolved desmethyl hydroxy glucuronide (phenolic) metabolite.
[d] The empirical formula for these structures is the same; therefore, this metabolite could not be identified based on accurate mass.
[e] Two desmethyl glucuronide metabolites were detected, one unresolved from CSCD659090.
[f] Includes a hydroxy glutathione metabolite unresolved from a desmethyl hydroxy glucuronide metabolite.

Table 15 (continued)

[g] A desmethyl hydroxy glucuronide (phenolic) metabolite was unresolved from a desmethyl β-hydroxycarbonyl metabolite.
[h] Four hydroxy glucuronide metabolites were detected, one unresolved from CSCD668404.
[i] A hydroxy glutathione metabolite was unresolved from an isomer of a desmethyl hydroxy glucuronide metabolite.
[j] Eleven components, none greater than 0.24% of the administered dose.
[k] Five components, none greater than 0.34% of the administered dose.
[l] Losses/gains on fractionation incorporate procedural losses. This is calculated from the sum of the per cent administered dose in each component subtracted from the per cent administered dose in sample.

Table 16. Metabolite profile in excreta of bile duct–cannulated rats following a single oral dose of [phenyl-U-^{14}C]sedaxane at the low dose of 1 mg/kg bw

Compound	% of administered dose (number of isomers)							
	Males				Females			
	Urine (0–48 h)	Faeces (0–48 h)	Bile (0.5–48 h)	Total excreta	Urine (0–48 h)	Faeces (0–48 h)	Bile (0.5–48 h)	Total excreta
Sedaxane	—	4.31	1.17	5.48	—	7.38	—	7.38
CSCD659087	0.86	—	1.47	2.33	1.62	—	1.27	2.89
CSCD668404	—	—	1.04[a]	1.04	0.54	0.57	—	1.11
CSCD658906	0.46	0.38	1.22	2.06	0.64	0.27	0.79	1.70
CSCD659089	—	0.33	—	0.33	—	0.18	—	0.18
CSCD659090	—	—	1.19[b]	1.19	—	—	0.50[b]	0.50
Desmethyl β-hydroxycarbonyl	—	0.38 (1)	—	0.38	2.51 (1)[c]	—	—	2.51
Dihydroxy/β-hydroxycarbonyl[d]	0.75 (1)[d]	—	—	0.75	0.15 (1)[d]	—	—	0.15
Carboxylic acid	0.43 (1)	—	—	0.43	0.13 (1)	—	—	0.13
Hydroxy β-hydroxycarbonyl	0.29 (1)	—	—	0.29	0.33 (1)	—	—	0.33
Hydroxy cysteine conjugate	—	—	—	—	—	—	2.33 (1)	2.33
Desmethyl glucuronide	—	—	1.02 (2)[e]	1.02	—	—	0.65 (2)[e]	0.65
Desmethyl hydroxy glucuronide	1.12 (2)	—	40.23 (4)[f]	36.37	0.26 (2)[g]	—	40.87 (5)[f]	41.13
Hydroxy glucuronide	0.32 (1)	—	36.93 (4)[h]	37.25	0.85 (1)	—	32.49 (3)	33.34
Hydroxy glutathione conjugate	—	—	(1)[i]	—	—	—	(1)[i]	—
Post-extraction solids	NA	0.20	NA	0.20	NA	0.10	NA	0.10
Total identified	4.23	5.40	84.27	93.90	7.03	8.40	78.90	94.33
Total unidentified	1.57	0.00	1.06	2.63[j]	0.46	0.00	3.21	3.67[k]
Total accounted for	5.80	5.60	85.33	96.73	7.49	8.50	82.11	98.10
Losses/gains[l]	0.70	0.10	−4.23	−3.43	0.61	0.10	−3.51	−2.80
Total	6.50	5.70	81.10	93.30	8.10	8.60	78.60	95.30

From Green (2009)
NA, not applicable
[a] Includes an unresolved hydroxy (phenolic) glucuronide metabolite.
[b] Includes an unresolved desmethyl glucuronide metabolite.
[c] Includes an unresolved desmethyl hydroxy glucuronide (phenolic) metabolite.
[d] The empirical formula for these structures is the same; therefore, this metabolite could not be identified based on accurate mass.
[e] Two desmethyl glucuronide metabolites were detected, one unresolved from CSCD659090.
[f] Includes a hydroxy glutathione metabolite unresolved from a desmethyl hydroxy glucuronide metabolite.
[g] Two desmethyl hydroxy glucuronide metabolites were detected, one unresolved from a desmethyl β-hydroxycarbonyl metabolite.
[h] Four hydroxy glucuronide metabolites were unidentified, one unresolved from CSCD668404.
[i] A hydroxy glutathione metabolite was unresolved from a desmethyl hydroxy glucuronide metabolite.
[j] Eight components, none greater than 1.06% of the administered dose.
[k] Five components, none greater than 2.73% of the administered dose.
[l] Losses/gains on fractionation incorporate procedural losses. This is calculated from the sum of the per cent administered dose in each component subtracted from the per cent administered dose in sample.

Sedaxane was extensively metabolized by rats via demethylation, hydroxylation, oxidation and conjugation reactions, resulting in an array of hydroxylated metabolites and metabolites formed by cleavage of the terminal cyclopropyl moiety. An equivalent range of metabolites of desmethyl sedaxane was formed. The major metabolites were identified as the *trans-para*-phenol CSCD658906 and the desmethyl *trans-para*-phenol CSCD659087, which, together with the equivalent *cis-para*-phenol isomers CSCD659090 and CSCD668404, accounted for approximately half the administered dose. Glucuronic acid, sulfate and glutathione conjugates were formed (Green, 2009).

On the basis of these studies, the metabolic pathway shown in Figure 2 was proposed.

Figure 2. Overall summary of the biotransformation pathway of sedaxane (SYN 524464) in rat

2. Toxicological studies

2.1 Acute toxicity

Studies on the acute toxicity of sedaxane are summarized in Table 17.

Table 17. Summary of acute toxicity of sedaxane

Species/strain	Route	Sex	Parameter evaluated	Result	Ratio (%) of *trans* to *cis* isomers	Reference
Rat / HanRcc:WIST	Oral	F	LD_{50}	5000 mg/kg bw	83.0:12.3	Arcelin (2008)
Rat / HanRcc:WIST	Dermal	M + F	LD_{50}	> 5000 mg/kg bw	83.0:12.3	Arcelin (2007a)
Rat / HanRcc:WIST	Inhalation	M + F	4 h LC_{50}	> 5.244 mg/l	83.0:12.3	Decker (2008)
Rabbit / NZW	Dermal	M + F	Local irritancy	Non-irritant	83.0:12.3	Arcelin (2007b)
Rabbit / NZW	Ocular	M + F	Local irritancy	Mild irritant	83.0:12.3	Arcelin (2007c)
Mouse / CBA/Ca	Intradermal and topical	M	Local lymph node assay	Non-sensitizer	83.0:12.3	Pooles (2007)

F, female; LC_{50}, median lethal concentration; LD_{50}, median lethal dose; M, male; NZW, New Zealand White

(a) Oral administration

Rats

In a range-finding acute oral toxicity study, one female HanRcc:WIST (SPF) rat was administered sedaxane (purity 95.3%) orally via gavage at 5000 mg/kg bw. The treated female died shortly after dosing. In a definitive study, 10- to 11-week-old female rats were dosed by gavage with sedaxane in 0.5% carboxymethylcellulose at 175, 550, 1750 or 5000 mg/kg bw (one, one, four and seven females per dose, respectively). All surviving rats were killed and necropsied 15 days after dosing.

No deaths occurred at 175, 550 or 1750 mg/kg bw. At 5000 mg/kg bw, four out of seven rats were killed in extremis on day 1, and one rat died on day 2. Clinical signs recorded included ruffled fur, hunched posture, sedation, poor coordination and ventral recumbency in rats at 5000 or 1750 mg/kg bw. Deep respiration, rales, salivation and bradypnoea were recorded at 5000 mg/kg bw. The single rat given 550 mg/kg bw showed slightly ruffled fur or a hunched posture from 1 hour after dosing up to day 3. The 175 mg/kg bw treated female (one female treated with this dose) had slightly ruffled fur for approximately 0.5–5 hours, a hunched posture for 2–3 hours after the dosing and slight sedation at the 3-hour evaluation. Body weights were normal. At necropsy, a yellowish discoloration of the jejunum was recorded in one 5000 mg/kg bw dose rat killed in extremis, and pale, discoloured lungs were observed in another.

Based on the results of this study, it is estimated that the median lethal dose (LD_{50}) of sedaxane was 5000 mg/kg bw in female rats (Arcelin, 2008).

(b) Dermal application

Rats

A group of five male and five female HanRcc:WIST (SPF) rats was treated with sedaxane (lot no. SMU6LP006; purity 95.3%) at 5000 mg/kg bw by dermal application. In this study, a single animal of each sex was treated first. No deaths and no severe local effects or systemic symptoms were

observed after the 24-hour exposure. Therefore, the test was completed using the remaining four male and four female rats at a dose level of 5000 mg/kg bw with an exposure period of 24 hours. Sedaxane was applied undiluted and moistened with approximately 1 ml purified water for treatment to an area of skin from which hair had been clipped and covered with a semi-occlusive dressing for 24 hours. The rats were observed for 14 days after treatment, after which they were killed and subjected to a macroscopic examination.

All rats survived until the end of the study. No treatment-related effects on mortality, clinical signs or body weight or at gross necropsy were seen.

The acute dermal LD_{50} of sedaxane after a single dermal administration to rats of both sexes was greater than 5000 mg/kg bw (Arcelin, 2007a).

(c) Exposure by inhalation

Rats

A group of five male and five female HanRcc:WIST (SPF) rats 9–10 weeks of age was exposed for 4 hours by nose-only, flow-past inhalation to sedaxane (lot no. SMU6LP006; purity 95.3%) at a gravimetrically determined mean aerosol concentration of 5.244 mg/l air. The particle size distribution of the test atmosphere was analysed twice during the exposure period. The rats were observed for 15 days (including the exposure day as test day 1 of 15). Clinical observations and body weights were recorded throughout the study. The rats were necropsied at the end of the 15-day observation period.

The mass median aerodynamic diameter (MMAD) was 2.97–3.02 µm. There were no deaths and no macroscopic pathological findings. Transient clinical signs including bradypnoea and breath sounds (rales), decreased spontaneous activity, hunched posture and ruffled fur, and transient, slight retardation in body weight gain or marginal to moderate body weight loss in all rats were attributed to sedaxane, although slight physical stress during restraint in the exposure tubes may have contributed to the effects on body weight.

The acute inhalation LC_{50} of sedaxane after a 4-hour exposure in male and female rats was estimated to be greater than 5.244 mg/l air (Decker, 2008).

(d) Dermal irritation

The primary skin irritation potential of sedaxane (lot no. SMU6LP006; purity 95.3%) was investigated. A dose of 0.5 g sedaxane was applied to the shaved intact left flank of each of three young adult New Zealand White rabbits (one male and two females). After 4 hours of semi-occlusive treatment, the dressing was removed, and skin reactions were scored after 1, 24, 48 and 72 hours. The primary irritation index was calculated by totalling the mean cumulative scores at 24, 48 and 72 hours and then dividing by the number of data points (Draize, Woodward & Calvery, 1944; Draize, 1959).

No clinical signs were observed, and there were no signs of corrosion, irritation or staining of the skin in any of the rabbits throughout the 72 hours of observation. The primary irritation index was 0.0 (on a scale of 0.0–8.0).

According to Draize classification criteria, sedaxane was not an irritant to rabbit skin (Arcelin, 2007b).

(e) Ocular irritation

The primary eye irritation potential of sedaxane (purity 95.3%) was investigated according to Organisation for Economic Co-operation and Development Test Guideline No. 405. Sedaxane was applied by instillation of 0.1 g into the left eye of each of three young New Zealand White rabbits (one male and two females).

The instillation of sedaxane into the eye resulted in mild, early-onset and transient conjunctival reddening and chemosis. The individual mean scores for the conjunctivae were 0.33, 0.67 and 0.33 for reddening for each of the three rabbits and 0.00 for chemosis. These effects were no

longer observed 72 hours after treatment. No corrosion, staining of the eyes or any other abnormal ocular findings were observed in any rabbit at any examination time. No clinical signs were observed.

Under the conditions of this study, sedaxane was mildly irritating to rabbit eye (Arcelin, 2007c).

(f) Dermal sensitization

A sample of sedaxane (lot no. SUM6LP006; purity 95.3%) was assessed for its skin sensitization potential in CBA/Ca mice using the local lymph node assay. The assay compares the level of T lymphocyte proliferation in the lymph nodes draining the site of chemical application in mice treated with a chemical with T lymphocyte proliferation at a similar site in control group mice, by measuring the amount of radiolabelled thymidine incorporated into the dividing cells. The criterion for a positive response is that one or more of the concentrations tested should elicit a 3-fold or greater increase in isotope incorporation relative to the vehicle control group. The assay is able to identify those materials that elicit responses in standard guinea-pig tests for skin sensitization (Kimber et al., 1994). The application of sedaxane at concentrations of 10%, 25% and 50% weight per weight in acetone/olive oil (4:1) resulted in an isotope incorporation ratio that did not exceed 1.12, whereas a positive control treatment with hexylcinnamaldehyde, 15% volume per volume, resulted in an isotope incorporation ratio of 5.67.

Under the conditions of this study, sedaxane was not a skin sensitizer (Pooles, 2007).

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

Groups of five male and five female CD-1 mice were fed diets containing 0, 1000, 5000 or 7000 ppm sedaxane (lot no. S01F002249U; purity 98.2%; ratio of isomers 83.4% *trans* to 14.8% *cis*) for a period of at least 28 days. The mean compound intakes were 0, 178, 920 and 1268 mg/kg bw per day for males and 0, 248, 1150 and 1800 mg/kg bw per day for females at 0, 1000, 5000 and 7000 ppm, respectively. The mice were monitored regularly for viability and for signs of ill-health or reaction to treatment. Body weights and feed consumption were measured and recorded at predetermined intervals from pretrial until the completion of treatment. Blood samples were collected prior to terminal necropsies during week 5 for laboratory investigations. All animals were subjected to a detailed necropsy examination after the completion of treatment. A limited number of tissues from all mice in the 0 and 7000 ppm dose groups were taken for histological evaluation.

Stability, homogeneity and concentrations in the diet were acceptable. There were no differences in body weight and body weight gain that were attributable to treatment. There were no effects on body weight or feed consumption of males or females at dose levels up to 7000 ppm. A slight increase in haemoglobin value (4%) was observed in males at 7000 ppm that was not considered to be toxicologically significant. Statistically significant decreases in calcium levels compared with controls were observed in males at 5000 and 7000 ppm and in females at 7000 ppm. A 64% higher triglyceride concentration was observed in males at 7000 ppm. The blood chemistry changes were not accompanied by any corroborating histopathology. In addition, these changes were not detected in mice of the 7000 ppm group in the 90-day oral toxicity study described below. Therefore, they were not considered to be toxicologically significant. No treatment-related histopathological changes were observed.

The no-observed-adverse-effect level (NOAEL) was 7000 ppm (equal to 1268 mg/kg bw per day), the highest dose tested (Shearer & Robsertson, 2008).

In a dose-finding study preparatory to a carcinogenicity study, groups of 10 male and 10 female CD-1 mice were fed diets containing sedaxane (lot no. SMU6LP006; purity 95.3%; ratio of isomers 83.0% *trans* to 12.3% *cis*) at a concentration of 0, 500, 3500 or 7000 ppm (equal to 0, 80, 566

and 1167 mg/kg bw per day for males and 0, 112, 810 and 1455 mg/kg bw per day for females, respectively) for a period of at least 90 days. Mice were monitored regularly for viability and for signs of ill-health or reaction to treatment. Body weights and feed consumption were measured and recorded at predetermined intervals from pretrial until the completion of treatment. Blood samples were collected prior to terminal necropsies during week 14 for laboratory investigations. All mice were subjected to a detailed necropsy examination after the completion of treatment. Selected tissues from all mice in the 0 and 7000 ppm dose groups were taken for histological evaluation.

Stability, homogeneity and concentrations in the diet were acceptable. There were no treatment-related clinical signs throughout the study. Males at 7000 ppm had lower body weight and body weight gain throughout the treatment period (Table 18). Lower body weights were also noted in males at 500 or 3500 ppm, but these values were not statistically significant. The differences at 500 and 3500 ppm were not considered to be treatment related because the value in the control group was high compared with the historical control value. Lower feed utilization in males at 7000 ppm was observed along with the lower body weights. Statistically significantly lower white blood cell and lymphocyte counts were noted in all male treated groups, but with no indication of a dose-related response. However, the control values for white blood cells and lymphocytes were considered to be high (10.19×10^9/l and 8.28×10^9/l, respectively) when compared with mean historical control values (7.35×10^9/l and 5.61×10^9/l, respectively, based on eight relevant studies in male mice conducted from 2001 to 2004). There was no decrease in spleen weight, and there was no histopathological finding indicating immunosuppression in the bone marrow, spleen, lymph nodes or thymus. Therefore, these lower levels in haematology were not considered to be treatment related.

Table 18. Intergroup comparison of selected organ weights (absolute and adjusted) and clinical chemistry values of mice treated orally with sedaxane for 90 days

	Males				Females			
	0 ppm	500 ppm	3500 ppm	7000 ppm	0 ppm	500 ppm	3500 ppm	7000 ppm
Terminal body weight ± SD (g)	48 ± 7	45 ± 6	44 ± 4	43 ± 3	32 ± 5	31 ± 3	29 ± 2	34 ± 7
Alkaline phosphatase (IU/l)	62	52	47*	44**	62	60	56	56
Total bilirubin (mmol/l)	3.6	3.6	2.9	2.1**	2.4	2.4	2.8	2.4
Liver (g)	2.24	2.28	2.26	2.49	1.71	1.69	1.66	2.02
Liver, adjusted[a] (g)	2.05	2.29*	2.30*	2.61**	1.69	1.73	1.80	1.85
Kidney (g)	0.720	0.694	0.700	0.730	0.404	0.408	0.384	0.369
Kidney, adjusted[a] (g)	0.695	0.696	0.706	0.748	0.401	0.412	0.400	0.351**
Testis (g)	0.25	0.27	0.29	0.31**	—	—	—	—
Testis, adjusted[a] (g)	0.25	0.27	0.29*	0.31**	—	—	—	—

From Shearer & Foster (2008)

IU, international unit; SD, standard deviation; * $P < 0.05$; ** $P < 0.01$ (Dunnett's test, two-sided)

[a] Organ weights were adjusted for terminal body weights.

Blood chemistry analysis indicated statistically significant reductions in alkaline phosphatase activity in male mice of 29% and 24% at 7000 and 3500 ppm, respectively, and in total bilirubin level of 42% at 7000 ppm (Table 18). There were no statistically significant changes in blood chemistry of female mice. Absolute testis weights were significantly higher by 24% in the 7000 ppm group, but there were no other absolute organ weight changes. Relative to body weight, liver weights were increased in male mice of the 7000, 3500 and 500 ppm groups by 27%, 12% and 12%, respectively,

whereas there were no changes in females. The changes in relative liver weights in male mice appear to be attributable to the lower values for terminal body weight, because brain weights were not changed (male mice, 0 ppm, 0.51 g; 7000 ppm, 0.51 g). There was little change in liver weight relative to brain weight at increasing dose. Female mice of the 7000 ppm group had lower relative kidney and heart weights, but only after adjustment for body weight. As these changes were slight or lacked associated histopathological findings, these clinical chemistry and organ weight differences were not considered to be toxicologically significant (Shearer & Foster, 2008).

The NOAEL for this 90-day oral toxicity study in mice was 3500 ppm (equal to 566 mg/kg bw per day), based on a decrease in body weight gain throughout the study in males at 7000 ppm (equal to 1167 mg/kg bw per day) (Shearer & Foster, 2008).

Rats

Two short-term studies were conducted in rats. In the first study (Noakes, 2007), groups of 12 male and 12 female HsdRccHan:WIST rats were fed diets containing sedaxane (lot no. S01F002249U; purity 98.2%; ratio of isomers 83.4% *trans* to *cis* 14.8%) at a concentration of 0, 250, 1000 or 4000 ppm (equal to 0, 18.6, 72.9 and 299.6 mg/kg bw per day for males and 0, 21.4, 85.7 and 315.3 mg/kg bw per day for females, respectively) for 90 consecutive days. Clinical observations, body weights and feed consumption were measured throughout the study; a functional observational battery of tests and locomotor activity monitoring were performed during week 12. An ophthalmoscopic examination was performed on all rats before exposure and on control and high-dose rats in week 13. Urine samples collected during week 13 were analysed. At the end of the scheduled exposure period, the rats were killed and examined post mortem. Cardiac blood samples were taken for blood pathology, selected organs were weighed and specified tissues were taken for subsequent microscopic examination.

Stability, homogeneity and concentrations of sedaxane in the diet were acceptable. No treatment-related clinical signs were detected. No effects on functional observational battery parameters or locomotor activity were observed. At 4000 ppm, terminal body weights were lower in males (10%) and females (15%), and feed consumption by female rats was lower throughout the study. There were no treatment-related changes in ophthalmoscopic examination, functional observational battery assessments and motor activity, or macroscopic findings in any treated groups. Haemoglobin, haematocrit and red blood cell counts were slightly low for females at 4000 ppm. Prothrombin time was slightly, but significantly, longer (4% and 8%, respectively) for males at 1000 and 4000 ppm, and platelet counts were slightly, but significantly, higher (11%) for females at 4000 ppm. In blood chemistry, plasma triglyceride concentrations were higher in male rats at 1000 and 4000 ppm (40% and 63%, respectively), whereas female rats at 4000 ppm had higher triglycerides (64%) and total cholesterol (29%). At 4000 ppm, total protein was slightly increased in males (6%) and females (5%), and albumin was increased in males (5%). In urine analysis, reduced urine volume with high specific gravity was observed for males at 1000 and 4000 ppm. Urinary pH was also marginally, but significantly, lower than the control mean value in males at 4000 ppm (6.86 versus 6.54).

Absolute and adjusted liver weights were higher than control values in males at 4000 ppm (26% and 40%, respectively), in females at 4000 ppm (15% and 27%, respectively) and in females at 1000 ppm (13% and 11%, respectively). Adjusted liver weights only were increased by 8% in males at 1000 ppm. The liver weight changes observed in both sexes at 4000 ppm were accompanied by centrilobular hypertrophy and increased pigmentation. Changes in the weights of other organs occurred only at 4000 ppm. These consisted of increases in males of adjusted kidney weight (6%) and decreases in females of absolute weights of adrenals (14%), brain (4%), heart (15%) and kidney (12%). Neither heart nor kidney weight changes were accompanied by histopathology in these organs at 4000 ppm. Sporadic observation of changes in haematology, blood chemistry, urine analysis or relative organ weights at 1000 ppm were not considered to be toxicologically significant because of their small magnitude and the lack of accompanying histopathology that might point towards hepatotoxicity or renal toxicity at 1000 ppm. The treatment-related changes are shown in Tables 19 and 20.

Table 19. Summary of body weights and feed consumption in rats treated orally with sedaxane for 90 days

	Males				Females			
	0 ppm	250 ppm	1000 ppm	4000 ppm	0 ppm	250 ppm	1000 ppm	4000 ppm
Body weights (g)								
- week 2	196.1	198.3	197.2	185.6**	137.7	137.0	138.4	129.9**
- week 6	293.4	293.4	297.8	268.9**	188.8	184.7	191.2	164.6**
- week 10	343.5	341.0	344.9	310.8**	211.1	207.5	212.6	181.1**
- week 14	371.1	368.0	373.2	334.5**	221.2	218.7	221.4	189.8**
Feed consumption (g/rat per day)								
- week 1	21.2	21.8	21.1	17.3*	14.8	14.3	14.6	11.7**
- week 5	22.1	22.0	21.9	20.3*	17.4	16.2	17.0	13.2**
- week 9	20.5	20.5	20.1	19.7	16.5	15.9	16.2	12.8**
- week 13	19.2	19.8	19.1	19.2	15.2	14.8	15.0	12.9*
Haematological parameters								
Prothrombin time (s)	15.2	15.6	15.8**	16.4**	15.5	15.9	15.8	15.9
Platelets ($\times 10^9$/l)	893	890	897	936	909	955	939	1005*
Haemoglobin (g/dl)	15.2	15.2	15.1	14.9	15.4	14.5**	14.9	14.3**
Haematocrit (l/l)	0.473	0.470	0.473	0.469	0.475	0.445**	0.460	0.440**
Red blood cell count ($\times 10^2$/l)	8.47	8.60	8.61	8.36	8.25	7.86**	8.09	7.75**
Blood biochemical parameters								
Triglyceride (mmol/l)	1.41	1.72	1.98**	2.30**	0.89	0.81	0.97	1.46**
Cholesterol (mmol/l)	1.60	1.59	1.52	1.36	1.69	1.72	1.77	2.18**
Total protein (g/l)	60.1	59.7	61.3	63.7*	62.8	62.5	63.9	65.8*
Albumin (g/l)	33.1	33.3	33.7	34.8*	36.1	35.7	36.0	37.1

From Noakes (2007)
* $P < 0.05$; ** $P < 0.01$ (Student's *t*-test, two-sided)

The NOAEL in the first 90-day dietary study in rats was 1000 ppm (equal to 72.9 mg/kg bw per day), based on reduced body weight gain, liver toxicity (minimal centrilobular hepatocyte hypertrophy and pigmentation, evidence from blood chemistry of liver dysfunction, and increased prothrombin time) at 4000 ppm (equal to 299.6 mg/kg bw per day) (Noakes, 2007).

In the second short-term study, groups of 10 male and 10 female Han Wistar rats were fed sedaxane (lot no. SMU6LP006; purity 95.3%; ratio of isomers 83.0% *trans* to 12.3% *cis*) at 0, 300, 2000 or 4000 ppm (equal to 0, 24.8, 168.0 and 325.1 mg/kg bw per day for males and 0, 28.3, 186.0 and 349.8 mg/kg bw per day for females, respectively) for at least 90 days. The rats were monitored regularly for viability and for signs of ill-health or reaction to the diet. Detailed functional observations were performed once during treatment over a 2-week period (weeks 12/13). Grip strength was measured according to the method derived from Meyer et al. (1979). Pain was assessed by measurement of the tail flick response, using a technique based on the method devised by D'Amour & Smith (1941). Body weights and feed consumption were measured and recorded at predetermined intervals from pretrial until the completion of treatment. Ophthalmic assessments were undertaken on all animals pretrial and during week 13. Blood samples were collected at week 14 for haematology and blood chemical analysis. All rats were necropsied. Tissues from all rats in the 0 and

4000 ppm dose groups were subjected to comprehensive histological evaluation. In addition, the liver and thyroid were examined from all rats in the 300 and 2000 ppm dose groups.

Table 20. Summary of selected organ weights and histopathology in rats treated orally with sedaxane for 90 days

	Males				Females			
	0 ppm	250 ppm	1000 ppm	4000 ppm	0 ppm	250 ppm	1000 ppm	4000 ppm
Organ weights								
Liver (g)	12.3	13.1	13.3	15.5**	7.2	7.3	8.1**	8.3**
Liver, adjusted[a] (g)	11.9	11.9	12.8*	16.7**	7.0	7.1	7.8**	8.9**
Heart (g)	1.042	1.023	1.037	0.958	0.777	0.755	0.755	0.657**
Heart, adjusted[a] (g)	1.023	1.008	1.008	1.021	0.762	0.746	0.736	0.700*
Kidney (g)	2.09	2.06	2.12	2.03	1.36	1.36	1.35	1.20**
Kidney, adjusted[a] (g)	2.05	2.02	2.06	2.18*	1.32	1.33	1.30	1.31
Liver histopathology								
Hepatocellular hypertrophy (minimal)	0/12	0/12	0/12	12/12	0/12	0/12	0/12	10/12
Increased pigmentation (minimal)	0/12	0/12	0/12	6/12	0/12	0/12	0/12	10/12

From Noakes (2007)
* $P < 0.05$; ** $P < 0.01$ (Student's *t*-test, two-sided)
[a] Organ weight was adjusted for terminal body weight.

Stability, homogeneity and concentrations of sedaxane in the diet were acceptable. Agitated behaviour was noted occasionally in both sexes at 2000 and 4000 ppm and in two females in the 0 ppm group. This sign was not observed in the more comprehensive functional observational batteries, indicating that the signs in these groups were not treatment related. In male and female animals treated at 4000 ppm, body weight, body weight gain and feed consumption were significantly decreased (Table 21). In females, body weights and body weight gains were reduced at 2000 ppm (Table 21). Sedaxane had no effects on body weight or body weight gain at 300 and 2000 ppm in males or at 300 ppm in females and no effect on feed consumption at 300 or 2000 ppm in either sex. In detailed functional observational battery examinations, males at 4000 ppm showed a significant increase in hunched posture and piloerection in the observation arena. Decreases in forelimb grip strength in females at 2000 and 4000 ppm and hindlimb grip strength in females at 4000 ppm were observed (Table 21). These changes were considered to be indications of general toxicity rather than specific signs of neurotoxicity, because of the lack of any other neurological findings, including changes in motor activity or other functional observational battery parameters or histopathological findings in the nervous system.

In haematology, prothrombin time was significantly increased in both sexes at 4000 ppm, but no differences were detected in activated partial prothrombin time at any dose level; however, a lower number of samples per group were available for this parameter. A slightly lower (5%) statistically significant red blood cell count for male rats at 4000 ppm of 8.67×10^{12}/l was actually in the middle of the reported historical control range ($8.28–9.10 \times 10^{12}$/l), whereas the concurrent 0 ppm value of 9.15×10^{12}/l was at the upper end of this range. Consequently, the finding was interpreted as having no toxicological significance. At 4000 ppm, plasma gamma-glutamyl transferase was increased in both sexes, triglyceride and total protein concentrations were increased in males and plasma

cholesterol concentrations were increased in females. Haematological and blood biochemical changes are summarized in Table 22.

Table 21. Body weights and grip strength in functional observational battery in rats treated orally with sedaxane for 90 days (second study)

Parameter	Males				Females			
	0 ppm	300 ppm	2000 ppm	4000 ppm	0 ppm	300 ppm	2000 ppm	4000 ppm
Forelimb grip strength (g)	868	838	668*	697	810	708	632*	562**
Hindlimb grip strength (g)	604	595	575	551	572	595	507	459*
Body weight (g)								
- day 0	167	167	166	165	121	121	121	123
- day 7	217	217	212	197**	145	146	141	139
- day 28	289	284	274	263*	188	189	180	178*
- day 49	356	356	338	316*	217	217	200	198*
- day 70	392	397	382	351	237	235	215*	211*
- day 91	420	421	406	375	247	244	222*	218*

From Shearer & Foster (2009)
* $P < 0.05$; ** $P < 0.01$ (Dunnett's test, two-sided)

Table 22. Summary of haematology and blood biochemistry in rats treated orally with sedaxane for 90 days (second study)

Parameter	Males				Females			
	0 ppm	300 ppm	2000 ppm	4000 ppm	0 ppm	300 ppm	2000 ppm	4000 ppm
Haematology								
Prothombin time (s)	14.8	14.7	16.1	16.8*	15.2	15.2	15.4	16.5*
Red blood cell count ($\times 10^{12}$/l)	9.15	8.92	8.75	8.67*	8.32	8.03	8.01	8.20
Blood biochemistry								
Aspartate aminotransferase (IU/l)	83	92	67	67	77	71	69	63*
Gamma-glutamyl transferase (IU/l)	3	3	3	4*	3	3	3	5**
Cholesterol (mmol/l)	2.2	2.0	1.8	2.0	1.6	1.7	1.8	2.4**
Triglycerides (mmollL)	2.00	2.04	2.27	3.18**	1.58	1.26	1.98	2.12
Total protein (g/l)	70	69	71	73**	74	70	73	75

From Shearer & Foster (2009)
IU, international units; * $P < 0.05$; ** $P < 0.01$ (Dunnett's test, two-sided)

No treatment-related effects were observed at necropsy. In both male and female rats, increases were observed in absolute liver weights and liver weights adjusted for body weight at 4000 ppm and in adjusted liver weights at 2000 ppm. The liver weight changes in both male and female rats at 4000 ppm were accompanied by mild to moderate centrilobular hepatocyte hypertrophy and minimal to mild hepatocyte pigmentation. In addition, minimal to moderate diffuse thyroid follicular cell hypertrophy was observed in five males and one female at 4000 ppm (Table 23).

Table 23. Liver weights and selected histopathology in rats treated orally with sedaxane for 90 days (second study)

	Males				Females			
	0 ppm	300 ppm	2000 ppm	4000 ppm	0 ppm	300 ppm	2000 ppm	4000 ppm
Liver weight (g)	15.98	16.50	17.72	19.64**	9.18	9.03	10.17	11.85**
Liver weight, adjusted (g)[a]	15.38	15.71	17.73**	20.94**	8.46	8.51	10.60**	12.65**
Histopathology								
Liver								
Centrilobular hypertrophy (mild to moderate)	0/10	0/10	0/10	10/10***	0/10	0/10	0/10	10/10***
Hepatocyte pigment (minimal to mild)	0/10	1/10	0/10	8/10***	0/10	0/10	1/10	7/10**
Thyroid								
Follicular cell hypertrophy (minimal to moderate)	0/10	0/10	0/10	5/10**	0/10	0/10	0/10	1/10

From Shearer & Foster (2009)

* $P < 0.05$ (Fisher's exact probability test); ** $P < 0.01$ (organ weights, Dunnett's test, two-sided; histopathological findings, Fisher's exact probability test); *** $P < 0.001$ (Fisher's exact probability test)

[a] Organ weight was adjusted for terminal body weight.

The NOAEL in the second 90-day dietary study in rats was 300 ppm (equal to 28.3 mg/kg bw per day), based on decreases in forelimb grip strength in both sexes and body weight gains in females at 2000 ppm (equal to 168.0 mg/kg bw per day), supported by decreases in hindlimb grip strength, reduced body weight gain, liver toxicity and thyroid follicular cell hypertrophy at 4000 ppm (equal to 325.1 mg/kg bw per day) (Shearer & Foster, 2009).

Dogs

A small study was conducted as a guide to doses to be used in larger, 3- and 12-month studies with Beagle dogs. Groups of one male and one female Beagle dog were dosed orally (by capsule) with sedaxane (lot no. S01F002249U; purity 98.2%; ratio of isomers 83.4% *trans* to 14.8% *cis*) at 0, 50, 100 or 300 mg/kg bw per day for 4 weeks.

All dogs survived the scheduled treatment period. There were no treatment-related clinical signs at any dose level. There were no effects on ophthalmic examinations or other changes at veterinary examination and no effects on urine analysis parameters. Body weight and feed consumption reductions were recorded for the female dosed at 300 mg/kg bw per day during the first 2 weeks. Subsequently, the feed consumption improved, but body weight gain remained lower than the control value. An increase in liver weight associated with slight hepatocellular hypertrophy and vacuolation was observed in the male at 300 mg/kg bw per day. The female dog at 300 mg/kg bw per day had slight hepatocyte vacuolation. The female at 100 mg/kg bw per day also had minimal hepatocyte vacuolation. Although the number of dogs used was very small, there was a suggestion that hepatocellular vacuolation might be a treatment-related effect, as it was observed in both sexes at 300 mg/kg bw per day. Hepatocellular hypertrophy observed in one dog was not considered to be adverse, because there was no indication of hepatotoxicity (Jackson, 2008a).

Groups of four male and four female 5- to 6-month-old Beagle dogs were dosed orally by capsule with sedaxane (lot no. SMU6LP006; purity 95.3%; ratio of isomers 83.0% *trans* to 12.3% *cis*)

at 0, 50, 150 or 400 mg/kg bw per day for a period of 13 weeks. Clinical signs, body weight and feed consumption were recorded throughout the study. Ophthalmoscopic examination of the eye and detailed physical examination of all dogs were performed and blood and urine samples were collected for laboratory investigations at intervals during the study. Following completion of the scheduled treatment period, a detailed necropsy was performed on all dogs, and a number of selected organs were weighed. A comprehensive list of tissues and organs was prepared and examined microscopically.

All dogs survived the scheduled treatment period. No adverse clinical signs were observed that were considered to be related to treatment with the test compound. Slightly higher incidences of vomiting of feed (males and females) and vomiting of mucous (females only) at 400 mg/kg bw per day were considered not to be of toxicological significance. Cumulative body weight gains were lower in both sexes at 400 mg/kg bw per day throughout the study (Table 24). Cumulative body weight gain in females at 150 mg/kg bw per day was lower than the control values from day 71 to the end of the study. The body weights were higher than control values at the start of the study in males at 50 and 150 mg/kg bw per day, but were not different at termination. After week 1, feed consumption was consistently lower than the control group value in females at 400 mg/kg bw per day (Table 24).

Table 24. Cumulative body weight gain and feed consumption in dogs treated orally with encapsulated sedaxane for 90 days

	Males				Females			
	0 mg/kg bw per day	50 mg/kg bw per day	150 mg/kg bw per day	400 mg/kg bw per day	0 mg/kg bw per day	50 mg/kg bw per day	150 mg/kg bw per day	400 mg/kg bw per day
Cumulative body weight gain (kg)								
- day 8	0.03	0.11	−0.21	−0.62**	0.09	0.06	−0.30**	−0.57**
- day 15	0.37	0.28	0.33	−0.17	0.54	0.74	0.43	−0.17**
- day 22	0.87	0.52	0.62	0.15**	0.93	0.93	0.64	0.22*
- day 64	2.14	1.38	1.51	0.81**	2.07	1.85	1.45	1.01*
- day 71	2.40	1.51	1.66	1.02**	2.20	1.90	1.38*	1.07**
- day 78	2.60	1.70*	1.66*	0.99**	2.38	2.05	1.43*	1.33**
- day 85	2.58	1.66	1.65	1.14**	2.51	1.93	1.35**	1.15**
- day 92/93[a]	2.57	2.02	1.73	1.07**	2.56	1.77	1.22**	1.03**
Feed consumption (g)								
- pretest (day −6 to 1)	269	343*	331*	324	272	210**	247	246
- day 1	7.03	8.07	8.26	7.94	6.00	5.60	5.82	6.05
- day 8	7.06	8.18	8.05	7.32	6.10	5.66	5.53	5.48
- day 15	7.40	8.35	8.59	7.77	6.54	6.34	6.25	5.88
- day 71	9.43	9.58	9.92	8.96	8.20	7.50	7.20	7.12*
- day 92/93[a]	9.60	10.09	9.99	9.01	8.56	7.37*	7.04**	7.08*

From Jackson (2008b)

* $P < 0.05$; ** $P < 0.01$ (Dunnett’s test, two-sided)

[a] Day 92 (males), day 93 (females).

In haematological examination at termination, lower total leukocyte, lymphocyte and monocyte counts in females at 150 and 400 mg/kg bw per day and lower total leukocyte count in males at 400 mg/kg bw per day were observed, whereas no differences were observed at other time points. The lower leukocyte counts were not considered to be treatment related because they were relatively small and there was an absence of corresponding histopathological changes in bone marrow or lymph nodes.

The treatment-related difference in blood biochemistry was lower cholesterol in males at 400 mg/kg bw per day from week 4 to week 13 (Table 25). Lower cholesterol in males at 150 mg/kg bw per day was not considered to be treatment related, because only one male showed a slightly lower cholesterol level at this dose level at week 13.

Table 25. Cholesterol levels in male dogs treated orally with encapsulated sedaxane for 90 days

Week	Plasma cholesterol level (mmol/l)			
	0 mg/kg bw per day	50 mg/kg bw per day	150 mg/kg bw per day	400 mg/kg bw per day
Pretest	3.64	2.74**	3.26	3.27
1	2.73	2.11	2.57	2.83
4	3.05	2.31*	2.62	2.41*
8	3.49	2.53**	3.03	2.72*
13	3.43	2.34**	2.93*	2.53**

From Jackson (2008b)

* $P < 0.05$; ** $P < 0.01$ (Dunnett's test, two-sided)

There were no differences in ophthalmoscopy, veterinary examinations, organ weights or macroscopic findings between the control and treated groups. Microscopically, thyroid follicular cell hypertrophy (0, 1, 2, 1 in males; 0, 0, 0, 2 in females; at 0, 50, 150 and 400 mg/kg bw per day, respectively) was observed in some male dogs in all treated groups and in females at 400 mg/kg bw per day. In the absence of a dose–response relationship and considering the minimal severity, thyroid follicular cell hypertrophy is considered not to be treatment related.

The NOAEL in the 90-day oral (capsule) study in dogs was 50 mg/kg bw per day, based on lower cumulative body weight gain in females at 150 mg/kg bw per day (Jackson, 2008b).

Sedaxane (lot no. SMU6LP006; purity 95.3%; ratio of isomers 83.0% *trans* to 12.3% *cis*) was administered to four Beagle dogs of each sex per group orally, by capsule, at a dose level of 0, 15, 50 or 200 mg/kg bw per day for 52 weeks. Clinical signs, body weight and feed consumption were recorded throughout the study. Ophthalmoscopy and veterinary examinations were performed and blood and urine samples were collected for clinical laboratory investigations at intervals during the study. After the completion of treatment, all dogs were examined macroscopically and microscopically.

All dogs survived the treatment period. No treatment-related clinical signs were observed. Decreased feed consumption with a corresponding loss of body weight or low body weight gain was observed throughout the treatment period in the animals dosed at 200 mg/kg bw per day (Table 26). Feed intake was reduced from the 1st week of the treatment period. Following extension of the daily feeding period, feed intake improved transiently in the males but remained low in the females over the remainder of the treatment period.

Table 26. Body weights, cumulative body weight gains and feed consumption in dogs treated orally with encapsulated sedaxane for 1 year

	Males				Females			
	0 mg/kg bw per day	15 mg/kg bw per day	50 mg/kg bw per day	200 mg/kg bw per day	0 mg/kg bw per day	15 mg/kg bw per day	50 mg/kg bw per day	200 mg/kg bw per day
Body weight (kg)								
- day 1	9.25	8.84	9.18	8.96	7.37	7.14	7.75	7.29
- day 8	9.59	9.26	9.47	8.98	7.56	7.28	7.91	7.00
- day 92	10.83	11.02	11.25	9.59	9.01	8.70	9.63	7.40*
- day 365/366	11.50	12.54	12.71	10.54	10.07	10.16	10.98	8.21
Feed consumption								
- day 8	0.34	0.42	0.28	0.02*	0.19	0.15	0.16	−0.29**
- day 15	0.34	0.42	0.48	0.17	0.38	0.29	0.32	−0.29**
- day 50	1.26	1.62	1.48	0.51**	1.27	1.10	1.33	0.14**
- day 92	1.58	2.18	2.06	0.63*	1.64	1.56	1.88	0.11**
- day 169	1.67	2.55	2.52	1.26	1.82	2.20	2.64	0.60*
- day 239	1.76	3.06	2.96	1.68	2.02	2.26	3.14	0.69

From Braun (2009)

* $P < 0.05$; ** $P < 0.01$ (Dunnett's test)

In haematology, treatment-related changes were not observed. In blood chemistry, lower glucose levels and higher alkaline phosphatase activity were observed in males and females dosed at 200 mg/kg bw per day. Plasma cholesterol and phosphorus concentrations in males of the 200 mg/kg bw per day group were lower than those of controls (Table 27).

Liver weights, absolute and adjusted for terminal body weight, were increased in both sexes at 200 mg/kg bw per day (Table 28). Testes weights were lower than those of controls at 200 mg/kg bw per day. The testes weights in all groups except for one dog were within the range of historical control data of the laboratory (10.9–26.6 kg in 2000–2007, 11 studies), although they were in the lower range. A dog with the lowest weight of testes at 200 mg/kg bw per day showed inflammation with tubular atrophy and spermatogenic giant cells in the testes, and these changes were considered to be incidental. Treatment-related histopathological changes were not observed in other animals.

The NOAEL in the 1-year oral (capsule) study in dogs was 50 mg/kg bw per day, based on decreased feed consumption with corresponding initial body weight loss and lower body weight gain, minor changes in clinical biochemistry and higher liver weights in both sexes seen at 200 mg/kg bw per day (Braun, 2009).

(b) Dermal application

Rats

Groups of 10 male and 10 female HanRcc:WIST (SPF) rats were administered dermal doses of sedaxane (lot no. SMU6LP006; purity 95.3%; ratio of isomers 83.0% *trans* to 12.3% *cis*) at 0 (control), 100, 300 or 1000 mg/kg bw per day in bi-distilled water under semi-occlusive conditions for 6 hours/day, 5 days/week, over a period of 28 days. An area of skin of approximately 25 cm^2 was exposed. General clinical observations, detailed behavioural observations, body weight measurements and feed consumption measurements were made once weekly during the acclimatization and treatment periods. A functional observational battery, including grip strength and locomotor activity measurements, was conducted during the last week of treatment before the dermal administration for

that day. Eye examinations were performed in all rats during acclimatization and during week 4 in animals of the control and high-dose groups. Haematology and blood chemistry investigations were conducted. At necropsy, selected organs were weighed, and a range of tissues and organs was examined macroscopically and microscopically.

Table 27. Selected blood chemistry parameters in dogs treated orally with encapsulated sedaxane for 1 year

Parameter	Males				Females			
	0 mg/kg bw per day	15 mg/kg bw per day	50 mg/kg bw per day	200 mg/kg bw per day	0 mg/kg bw per day	15 mg/kg bw per day	50 mg/kg bw per day	200 mg/kg bw per day
Glucose (mmol/l)								
- pretest	5.75	6.07	6.11	5.77	6.22	5.95	5.96	5.52*
- week 13	5.49	5.78	5.76	5.01*	5.82	5.82	5.64	4.98
- week 26	5.25	5.70	5.57	4.93	5.58	5.55	5.80	5.03
- week 52	5.71	5.55	5.61	4.90*	5.89	5.89	6.01	5.10*
Alkaline phosphatase (U/l)								
- pretest	189	163	214	161	142	175	134	147
- week 13	112	99	145	155	96	108	86	118
- week 26	78	77	114	145*	72	79	79	114*
- week 52	66	81	112	180*	61	86	63	132**
Cholesterol (mmol/l)								
- pretest	2.82	2.39	2.75	2.43	2.37	2.56	2.30	2.42
- week 13	3.25	2.62	2.99	2.30	3.00	3.12	2.94	2.67
- week 26	3.31	3.02	3.46	2.46*	3.28	3.27	3.13	2.77
- week 52	3.52	3.27	3.63	2.35	3.90	3.93	3.15	3.32
Phosphorus (mmol/l)								
- pretest	2.28	2.27	2.28	2.28	2.03	2.09	2.15	2.24
- week 13	1.86	1.72	1.67	1.58*	1.71	1.61	1.57	1.58
- week 26	1.43	1.32	1.24	1.22*	1.15	1.03	1.13	1.25
- week 52	1.28	1.12	1.13	1.10	1.06	0.94	0.97	1.04

From Braun (2009).
U, unit; * $P < 0.05$; ** $P < 0.01$ (Dunnett's test, two-sided)

All rats survived the treatment. No sedaxane-related effects were recorded at the treated skin sites. General clinical observations, detailed behavioural observations, eye examination and functional observational battery investigations, including grip strength and locomotor activity measurements, revealed no effects related to sedaxane treatment. No treatment-related changes were observed in feed consumption, body weight, haematology, clinical biochemistry, urine analysis, organ weights or macroscopic and microscopic findings.

The NOAEL in the 28-day dermal toxicity study in rats was 1000 mg/kg bw per day, based on an absence of treatment-related changes at 1000 mg/kg bw per day, the highest dose tested (Sommer, 2009a).

Table 28. Organ weights in dogs treated orally with encapsulated sedaxane for 1 year

	Organ weight (g)							
	Males				Females			
	0 mg/kg bw per day	15 mg/kg bw per day	50 mg/kg bw per day	200 mg/kg bw per day	0 mg/kg bw per day	15 mg/kg bw per day	50 mg/kg bw per day	200 mg/kg bw per day
Liver weight	356	366	384	416*	325	365	343	338
Liver weight adjusted for body weight	357	362	379	423*	318	356	311	384
Testes weight	16.9	14.4	15.3	13.2*	—	—	—	—
Testes weight adjusted for body weight	16.9	14.4	15.2	13.3*	—	—	—	—

From Braun (2009)
* $P < 0.05$ (Dunnett's test)

2.3 *Long-term studies of toxicity and carcinogenicity*

Mice

Groups of 50 male and 50 female CD-1 mice were fed diets containing sedaxane (lot no. SMU6LP006; purity 95.3%; ratio of isomers 83.0% *trans* to 12.3% *cis*) at 0, 200, 1250 or 7000 ppm (equal to 0, 25, 157 and 900 mg/kg bw per day for males and 0, 29, 185 and 1001 mg/kg bw per day for females, respectively) for 80 weeks. The mice were monitored regularly for viability and for signs of ill-health or reaction to treatment. Body weights and feed consumption were measured and recorded at predetermined intervals from pretrial until the completion of treatment. Blood samples for haematology were collected from all surviving mice prior to terminal kill at week 80. Blood films were made from all surviving mice during week 52/53 and at week 80. All surviving mice were necropsied after the completion of treatment. Tissues from all mice were subjected to a comprehensive histological evaluation.

Homogeneity, stability and achieved concentration of sedaxane diets were acceptable. Body weights and body weight gains in both sexes at 7000 ppm were slightly lower than those of controls (Table 29). The maximum difference from control for body weight was 7% in males and 9% in females. Whereas feed consumptions in treated groups were comparable to the control group value, lower values of food utilization during two of the three measurement intervals at 7000 ppm were considered to be treatment related.

There were no treatment-related effects on haematological parameters in male or female mice. Liver weight was increased in males at 7000 ppm (+16%), but this change was considered to be an adaptive response due to the absence of any indication of hepatotoxicity on microscopic examination (Table 30). There were no treatment-related macroscopic or microscopic findings in any treated groups. A lower incidence of lymphomas in females was not considered to be toxicologically significant.

In male mice at 7000 ppm, the incidences of hepatocellular adenoma and adenomas and carcinomas combined were slightly, but statistically significantly, higher than those of the control group (Table 31). All other neoplastic alterations in the treated groups were comparable to controls in the laboratory.

The NOAEL in the 18-month dietary study in mice was 1250 ppm (equal to 157 mg/kg bw per day), based on reduced body weight and body weight gain in both sexes seen at 7000 ppm (equal to 900 mg/kg bw per day). A slightly increased incidence of hepatocellular adenomas and carcinomas combined was observed in male mice at the high dose in comparison with the control group incidence.

The NOAEL for equivocal carcinogenicity in mice was 1250 ppm (equal to 157 mg/kg bw per day) (Perry, 2010b).

Table 29. Summary of body weight changes in mice orally treated with sedaxane for 80 weeks

	Body weight / body weight change (g)							
	Males				Females			
	0 ppm	200 ppm	1250 ppm	7000 ppm	0 ppm	200 ppm	1250 ppm	7000 ppm
Body weight								
- week 0	34.0	34.2	33.0	33.3	24.0	23.8	24.2	23.9
- week 1	36.3	36.0	35.0*	34.9*	25.2	24.8	25.3	25.1
- week 3	38.5	38.6	37.7	37.5	27.2	26.7	27.1	26.3*
- week 13	48.0	47.3	46.8	45.6	32.8	31.8	32.6	31.1
- week 26	55.1	53.9	52.8	52.4	40.6	38.3	39.4	36.9*
- week 52	59.8	57.6	56.7	57.9	46.1	44.3	45.0	42.7
- week 80	61.3	59.6	58.7	57.6	49.4	48.2	49.2	45.6
Cumulative body weight change								
- weeks 0–1	2.2	1.8**	2.0	1.6**	1.2	1.1	1.2	1.2
- weeks 0–3	4.4	4.3	4.7	4.2	3.3	2.9	2.9	2.4**
- weeks 0–13	14.0	13.0	13.7	12.3	8.9	8.1	8.4	7.2
- weeks 0–80	27.5	25.7	25.8	24.7	25.5	24.3	25.1	21.7

From Perry (2010b)
* $P < 0.05$; ** $P < 0.01$ (Dunnett's test)

Table 30. Absolute and adjusted liver and adrenal weights in mice orally treated with sedaxane for 80 weeks

	Organ weight (g)							
	Males				Females			
	0 ppm	200 ppm	1250 ppm	7000 ppm	0 ppm	200 ppm	1250 ppm	7000 ppm
Liver, absolute	3.20	3.13	3.09	3.44	2.15	1.98	2.02	2.11
Liver, adjusted	3.04	3.13	3.15	3.53*	2.13	1.97	1.99	2.17
Adrenal, absolute	0.0057	0.0058	0.0051	0.0061	0.0084	0.0095	0.0101*	0.0100
Adrenal, adjusted	0.0056	0.0057	0.0052	0.0061	0.0084	0.0095	0.0101*	0.0100

From Perry (2010b)
* $P \leq 0.05$ (Dunnett's test)

Rats

Four groups of 52 male and 52 female Han Wistar rats were assigned to the carcinogenicity study and administered diets containing sedaxane (lot no. SMU6LP006; purity 95.3%; ratio of isomers 83.0% *trans* to 12.3% *cis*) at 0, 200, 1200 or 3600 ppm (equal to 0, 11, 67 and 218 mg/kg bw per day for males and 0, 14, 86 and 261 mg/kg bw per day for females, respectively) for 104 weeks. A 52-week toxicity study comprising an additional four groups of 12 male and 12 female rats was combined with the 104-week component to examine clinical observations, body weight, feed consumption, haematology, coagulation potential, clinical chemistry, organ weights, and gross and microscopic lesions. Additionally, carcinogenicity study rats underwent eye examinations and had urine samples taken from them for analysis. The 52-week toxicity study rats underwent a detailed

Table 31. Incidence of hepatocellular adenoma and carcinoma in male mice orally treated with sedaxane for 80 weeks

	Incidence of finding[a]				Historical control incidence	
	0 ppm	200 ppm	1250 ppm	7000 ppm	Laboratory (range)[b]	RITA (range)[c]
Adenoma						
Intercurrent	1/48	2/45	1/45	3/48	Four studies including concurrent study. 14/50, 5/50, 11/50, 30/150 (range, 10–28%)	0.0–13.6%
Terminal kill	6/48	7/45	9/45	12/48		
Total (%)	7/48 (15%)	9/45 (20%)	10/45 (22%)	15/48* (31%)		
	0 ppm	*Low*	*Mid*	*High*		
From concurrent study (%)	14/50 (28%)	17/50 (34%)	17/50 (34%)	13/50 (26%)		
Carcinoma						
Intercurrent	1/48	0/45	0/45	4/48	Four studies including concurrent study. 3/50, 5/50, 3/50, 11/150 (range, 6–10%)	4.0–22.0%
Terminal kill	4/48	5/45	3/45	6/48		
Total (%)	5/48 (10%)	5/45 (11%)	3/45 (7%)	10/48 (21%)		
	0 ppm	*Low*	*Mid*	*High*		
From concurrent study (%)	3/50 (6%)	10/50 (20%)	3/50 (6%)	4/50 (8%)		
Adenoma and carcinoma combined[d]						
Combined adenoma and carcinoma (%)	9/48 (19%)	13/45 (29%)	12/45 (27%)	19*/48 (40%)		

From Perry (2010b)

RITA, Registry of Industrial Toxicology Animal-data; * $P < 0.05$ (Fisher's exact test)

[a] Number of tumour-bearing animals/number of animals examined, excluding those that died before week 49.

[b] Laboratory historical control data = four studies, all started in 2007.

[c] RITA historical control data are shown for studies of 18–19 months' duration.

[d] Number of animals bearing both an adenoma and a carcinoma were 3, 1, 1 and 6 at 0, 200, 1250 and 7000 ppm, respectively.

functional observational battery assessment at week 51/52. Grip strength was measured according to the method derived from Meyer et al. (1979). Pain was assessed by measurement of the tail flick response, using a technique based on the method devised by D'Amour & Smith (1941). All surviving carcinogenicity and toxicity study rats were killed at 104 or 52 weeks of treatment, respectively.

Dietary concentrations were measured according to the methodology developed by Currie (2007). Homogeneity, stability and achieved concentrations of sedaxane in the rat diet were acceptable. There were no treatment-related differences in mortality or clinical signs between the control and treated groups in both sexes. At 52 weeks, no treatment-related effects were observed in detailed clinical observations, motor activity or any other functional observational battery parameters. There were no ophthalmoscopic findings associated with treatment. There was clearly a treatment-related effect on body weight in both sexes at 3600 ppm throughout the study (Table 32). At termination, the body weight gains at 3600 ppm were depressed by 24% and 50% in males and females, respectively.

Table 32. Body weights and cumulative body weight changes in rats treated orally with sedaxane for 2 years

Parameter	Body weight / body weight change (g)							
	Males				Females			
	0 ppm	200 ppm	1200 ppm	3600 ppm	0 ppm	200 ppm	1200 ppm	3600 ppm
Body weight								
- week 0	148.5	150.6	145.6	149.3	129.4	129.9	129.9	132.5
- week 1	197.6	201.3	191.0	182.7**	152.7	152.5	150.4	144.9**
- week 3	260.6	267.5	254.8	232.4**	183.4	185.0	180.5	167.9**
- week 13	381.0	398.6*	373.7	336.6**	235.5	238.0	232.2	205.4**
- week 26	438.5	453.7	425.4	379.2**	259.0	259.0	249.0**	219.4**
- week 30	450.7	467.7	438.9	394.1**	261.1	262.8	254.8	224.2**
- week 32	460.3	473.8	440.4	383.7**	264.8	266.5	257.4	209.3**
- week 34	467.0	478.1	448.2	404.0**	268.3	269.4	258.6*	228.1**
- week 52	518.8	537.9	506.5	450.0**	292.9	295.5	281.1	240.2**
- week 104	613.5	657.2	590.5	503.6**	392.5	389.9	362.2*	264.1**
Body weight change								
- weeks 0–1	49.1	50.7	45.4**	33.3**	23.2	22.6	20.5**	12.4**
- weeks 0–3	112.1	116.8	109.2	83.0**	54.0	55.1	50.6	35.4**
- weeks 0–13	232.5	247.9*	228.0	187.3**	106.1	108.1	102.2	72.9**
- weeks 0–52	370.3	387.3	360.9	300.7**	163.5	165.6	151.1**	108.1**
- weeks 0–104	464.9	509.7*	447.9	355.7**	262.6	259.2	232.7*	132.4**

From Perry (2010a)
* $P < 0.05$; ** $P < 0.01$ (Dunnett's test, two-sided)

Female rats treated at 3600 ppm showed statistically significantly lower feed consumption compared with their respective controls (about 11–17%) throughout the treatment period, whereas males of the same treatment group had statistically significantly lower feed consumption only during weeks 1–7 (< 10%). Both males and females of the 3600 ppm sedaxane group had statistically significantly reduced feed utilization during weeks 1–4 and 9–13. The feed consumption and feed utilization profiles of males and females receiving 200 or 1200 ppm closely resembled those of their respective controls.

Prothrombin time was higher in males of the 3600 ppm group at multiple measurement times, but no other haematological differences between control and treatment groups were consistently observed. Blood chemistry analysis (Table 33) showed that plasma protein, albumin and globulin levels were increased in males and females at 3600 ppm. Slightly higher plasma gamma-glutamyl transferase values were observed in males at 3600 ppm at 104 weeks. Glucose and phosphate levels were high in males at 3600 ppm at several time points, and increases in plasma glucose were observed at 1200 ppm at two time points. Cholesterol was higher in females at 3600 ppm at weeks 14 and 27, but not later. Considering the small magnitude of these changes and lack of consistency, these changes are not considered to be treatment related except the increases in cholesterol in females at 3600 ppm. There were no treatment-related macroscopic findings at either 52 or 104 weeks.

Liver weights (adjusted for terminal body weight) were higher in both sexes in the 1200 ppm and 3600 ppm dose groups at both 52 and 104 weeks (Table 34).

Table 33. Summary of blood chemistry in rats orally treated with sedaxane for 2 years

Parameter	Males				Females			
	0 ppm	200 ppm	1200 ppm	3600 ppm	0 ppm	200 ppm	1200 ppm	3600 ppm
GGT (IU/l)								
- week 14	3	3	3	3**	3	3	3	4**
- week 27	3	3	3	4*	3	3	3	3
- week 52	3	3	3	3	3	3	3	3
- week 53	3	3	3	3*	3	3	3	3
- week 79	3	3	3	4**	3	5	3	3
- week 104	3	3	3	6**	3	3	3	3
Total protein (g/l)								
- week 14	67	69	69	71**	72	74	72	74
- week 27	68	70	71*	71**	73	73	72	73
- week 52	73	73	75	78**	78	77	80	79
- week 53	72	74	76*	77**	77	78	79	81*
- week 79	73	72	76	76	74	75	78	79*
- week 104	73	74	75*	76**	75	75	77	81**
Globulin (g/l)								
- week 14	24	26	25	27**	22	23	23	26**
- week 27	25	26	27	27	22	22	23	25**
- week 52	30	30	32*	32*	26	25	27	28
- week 53	29	30	31	32*	25	26	27	28**
- week 79	30	31	33	33	26	28	27	28
- week 104	31	34**	33**	33*	27	27	27	29
Cholesterol (mmol/l)								
- week 14	1.7	1.8	1.6	1.5	1.6	1.5	1.5	2.2**
- week 27	1.8	1.9	1.7	1.5*	1.8	1.5	1.6	2.1*
- week 52	2.3	2.3	2.2	1.9	2.1	2.1	2.1	2.6
- week 53	2.1	2.2	2.0	1.9	2.2	1.9	1.9	2.4
- week 79	2.8	2.6	2.7	2.2	2.2	2.3	2.2	2.4
- week 104	3.5	3.7	3.4	3.1	2.8	2.9	2.4*	2.6
Phosphate (mmol/l)								
- week 14	1.80	1.71	2.00*	2.00*	1.50	1.48	1.68	1.92**
- week 27	1.48	1.51	1.61	1.69	1.36	1.30	1.48	1.56
- week 52	1.36	1.32	1.42	1.43	1.21	1.17	1.28	1.21
- week 53	1.31	1.34	1.42	1.45	1.10	1.11	1.26	1.23
- week 79	1.24	1.46*	1.43*	1.49**	1.17	1.37	1.45*	1.40
- week 104	1.06	1.12	1.20**	1.30**	1.06	1.11	1.16	1.16
Glucose (mmol/l)								
- week 14	6.87	7.14	7.18	7.26	7.09	7.30	7.55	7.28
- week 27	6.98	7.43	7.75*	7.71	7.10	7.57	7.96*	7.13
- week 52	7.47	8.16	8.09	7.35	7.98	7.25*	7.12*	7.04**
- week 53	7.43	7.66	8.27	8.79**	7.64	7.67	8.15	7.27
- week 79	7.58	7.93	8.34*	8.32*	7.62	7.43	7.47	7.22
- week 104	6.60	6.99	7.25**	7.63**	5.92	6.62**	7.26**	6.87**

From Perry (2010a)
* $P < 0.05$; ** $P < 0.01$ (Dunnett's test, two-sided)

Table 34. Liver weights in rats orally treated with sedaxane for 2 years

	Liver weight (g)							
	Males				Females			
	0 ppm	200 ppm	1200 ppm	3600 ppm	0 ppm	200 ppm	1200 ppm	3600 ppm
52 weeks								
- absolute	16.30	17.09	19.29*	21.01**	9.21	9.33	9.89	10.39
- adjusted[a]	16.10	16.03	18.94**	22.63**	8.86	9.18	9.50	11.39**
104 weeks								
- absolute	18.57	19.40	20.21*	22.52**	12.72	13.24	13.07	12.17
- adjusted[a]	18.10	18.06	20.21**	24.20**	11.56	12.05	12.65**	14.64**

From Perry (2010a)
* $P < 0.05$; ** $P < 0.01$ (Dunnett's test, two-sided)
[a] Adjusted to terminal body weight.

The liver weight changes were accompanied at 52 weeks by centrilobular hepatocellular hypertrophy and pigmentation in males and females of the 3600 ppm dose group (Table 35). Treatment-related microscopic findings at 104 weeks included centrilobular hepatocyte hypertrophy in males at 1200 ppm and in both sexes at 3600 ppm. The incidence of hepatocellular pigmentation was increased in females at 3600 ppm. Eosinophilic cell foci were increased in males at 3600 ppm and in females in all treated groups. The increases in females in all treated groups were within the range of historical control data (7–32%) and considered to be attributed to low incidences in the control group, which were below the range of historical control data.

At 52 weeks, low incidences of thyroid follicular cell hypertrophy were observed in males at 1200 and 3600 ppm, without dose dependency (Table 35). At 104 weeks, an increase in focal follicular cell hyperplasia in the thyroid was noted in males at 3600 ppm. At 104 weeks, the incidences of colloid basophilia and desquamation of the follicular epithelium in the thyroid were increased in females at 1200 and 3600 ppm. Colloid basophilia was increased in males of the 3600 ppm dose group (Table 35).

Uterine adenocarcinoma was slightly, but significantly, increased at 3600 ppm (Table 36). The incidence of endometrial hyperplasia, a precancerous lesion, was not increased, and any changes indicating a higher ratio of estrogen to progesterone were not found in the ovary, uterus or vagina in short-term, long-term and reproductive toxicity studies of sedaxane. The incidence of endometrial adenocarcinoma in the control group was the lowest value of historical control data in the laboratory, and the incidence at 3600 ppm was close to the upper limit. The increase in uterine adenocarcinoma, however, was considered to be treatment related, because information about exclusion of the increase from the treatment was inadequate.

Decreased incidences of several neoplastic or non-neoplastic lesions, including mammary fibroadenoma, mammary lobular hyperplasia and chronic progressive nephropathy, in females at 3600 ppm were considered to be secondary effects corresponding to body weight depression at this dose level.

The NOAEL in the 104-week dietary study in rats was 200 ppm (equal to 11 mg/kg bw per day), based on increases in liver weight and histopathological changes in the liver (centrilobular hypertrophy) in males and in the thyroid in males and females and reduced body weight gain in females at 1200 ppm (equal to 67 mg/kg bw per day). The NOAEL for carcinogenicity in rats was 1200 ppm (equal to 86 mg/kg bw per day), based on uterine tumours in female rats (Perry, 2010a).

Table 35. Summary of histopathological changes in rats orally treated with sedaxane for 2 years

Non-tumour findings	Incidence of finding							
	Males				Females			
	0 ppm	200 ppm	1200 ppm	3600 ppm	0 ppm	200 ppm	1200 ppm	3600 ppm
52 weeks								
Liver								
Number examined	12	12	12	12	12	12	12	12
Basophilic cell focus, tigroid	1	1	0	0	5	1	0*	0*
Clear cell focus	11	6	9	4**	1	0	1	1
Hepatocyte hypertrophy, centrilobular	0	0	0	11***	0	0	0	12***
Hepatocyte pigment	0	0	0	7**	1	1	1	7*
Thyroid gland								
Number examined	12	12	12	12	12	12	12	12
Follicular cell hypertrophy	0	0	5*, +	4+	0	0	2	3
104 weeks								
Liver								
Number examined	52	52	52	52	52	52	52	52
Eosinophilic cell focus	8 (15.4%)	7 (13.5%)	15 (28.8%	25*** (48.1%)	2 (3.8%)	10* (19.2%)	12** (23.1%)	14** (26.9%)
Historical range at laboratory	16–86% (2002–2005, 5 studies)				7–32% (2002–2005. 5 studies)			
Basophilic cell focus, homogenous	2	0	1	0	1	0	2	1
Basophilic cell focus, tigroid	4	7	6	5	36	35	42	31
Clear cell focus	33	39	37	19*	12	16	14	12
Hepatocyte hypertrophy, centrilobular	0	0	8**	16***	0	0	1	38***
Hepatocyte pigment	0	1	0	1	2	3	1	15***
Thyroid gland								
Number examined	52	52	52	52	52	52	52	52
Desquamation, epithelial follicular	7	8	11	16	2	5	9*	14**
Basophilia, colloid	7	9	12	16+	3	6	11*	17***
Diffuse C-cell hyperplasia	27	27	24	10***	29	31	27	5***
Focal follicular cell hyperplasia	7	8	8	16+	0	4+	0	4+
Thymus								
Number examined	52	50	48	49	50	52	50	51
Hyperplasia, epithelial tubular	3	1	5	5	18	17	18	31*
Vagina								
Number examined	—	—	—	—	51	52	52	52
Mucification	—	—	—	—	15	22	16	3**
Mammary gland								
Number examined	43	43	45	41	52	50	51	52
Lobular hyperplasia	7	1	1*	4	34	34	32	21*

From Perry (2010a)

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (pairwise Fisher's exact test); + $P < 0.05$ (Mann-Whitney U-test). Other Mann-Whitney results for the indicated statistical results were similar to Fisher's test results.

Table 36. Incidence of and historical control data on uterine adenocarcinoma and adenoma in rats orally treated with sedaxane for 2 years

	Incidence of finding				Historical control data[a]	
	0 ppm	200 ppm	1200 ppm	3600 ppm	Laboratory	RITA
No. of uteri examined	52	52	52	52	2002–2005 (5 studies)	
Adenocarcinoma	0^	3 (4.7%)	2 (3.1%)	9** (17.3%)	0/50, 9/100, 10/99, 7/50, 21/110 (0–19%)	0–28%
Adenoma	0	0	1	0		

From Perry (2010a)

RITA, Registry of Industrial Toxicology Animal-data; ** $P < 0.01$ (pairwise Fisher's exact test); ^ $P < 0.05$ (positive trend by Peto trend test, groups 1–4; P-value for linear trend including groups 1–4 = 0.002; P-value for linear trend including groups 1–3 = 0.22)

[a] Laboratory historical control data = five studies, all started between 2002 and 2007; RITA historical control data are shown for studies of 22–25 months' duration.

2.4 Genotoxicity

Sedaxane was tested for genotoxicity in a range of assays, both in vitro and in vivo (Table 37). There was no evidence of mutagenic activity in a bacterial reverse mutation assay performed over a dose range up to 5000 µg/plate. In cultured mammalian cell assays, there was no evidence for clastogenicity or aneuploidy in primary cultures of human peripheral blood lymphocytes or of mutagenicity in mouse lymphoma L5178Y cells ($tk^{+/-}$ locus) over dose ranges that included moderately toxic concentrations. An in vivo study for the induction of micronuclei in polychromatic erythrocytes in the bone marrow of male NMRI mice showed no evidence of an effect of sedaxane at either 24 or 48 hours following a single oral dose of up to 2000 mg/kg bw. In a second study in vivo, there was no induction of unscheduled deoxyribonucleic acid (DNA) synthesis in hepatocytes isolated from male Sprague-Dawley rats treated orally with sedaxane at dose levels up to 2000 mg/kg bw and killed 2 hours later. Thus, sedaxane showed no evidence of genotoxicity or mutagenicity in any of these guideline studies, whereas significant responses to positive control treatment were observed in all studies.

Table 37. Summary of genotoxicity studies on sedaxane[a]

	Test system	Dose levels	Result	References
In vitro				
Bacterial reverse mutation (Ames test)	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537; *Escherichia coli* WP2(pKM101), WP2*uvrA*(pKM101)	3–5000 µg/plate	Negative (±S9)	Sokolowski (2009)
Test for clastogenicity in mammalian cells	Human peripheral blood lymphocytes; 4 or 22 h exposure	23.1–216.8 µg/ml	Negative (±S9)	Bohnenberger (2009)
Mammalian cell gene mutation	Mouse lymphoma L5178Y cells, $tk^{+/-}$ locus forward mutation	6.9–110 µg/ml	Negative (±S9)	Wollny (2009)
In vivo				
Mouse bone marrow micronucleus test	NMRI mouse bone marrow from 6 male mice per dose, obtained 24 or 48 h after exposure	500–2000 mg/kg bw	Negative	Reichenbach (2010)
Unscheduled DNA synthesis	Rat hepatocytes; 2 or 16 h exposure	667–2000 mg/kg bw	Negative	Durward (2009)

S9, 9000 × *g* supernatant fraction of rat liver homogenate

[a] Lot no. SMU6LP006 95.3% (ratio of isomers 83.0% *trans* to 12.3% *cis*) used in all assays.

2.5 *Reproductive and developmental toxicity*

(a) Multigeneration studies

Rats

A dose range–finding reproductive toxicity study in rats was performed. Sedaxane (lot no. SMU6LP0006; purity 95.3%; ratio of isomers 83.0% *trans* to 12.3% *cis*) was administered to four groups of F_0 generation male and female rats (Crl:WI (Han)) (10 of each sex per group) in the diet at a nominal dose level of 0, 500, 1500 or 3600 ppm for 10 weeks before pairing through to weaning of the F_1 generation. Toxicity of sedaxane was also investigated in the F_1 generation when continuously available in the diet from birth up to day 35 of age. After the F_1 generation pups were weaned (day 28 of age), up to three males and three females were selected from each litter from the 0, 500 and 1500 ppm diet groups and retained until day 35 of age. High-dose (3600 ppm) pups were not retained after day 28 of lactation. The F_1 generation rats received sedaxane in the diet at the same nominal dose level as the F_0 generation from which they were selected. All rats were examined for effects on general condition, body weight and feed consumption. Organ weights were recorded at necropsy for all F_0 generation rats, for one male and one female pup per litter not selected for retention after day 28, and for one male and one female pup per litter selected for rearing on day 35 of age.

Mean body weight gain was statistically significantly lower than the control value for males given 3600 ppm throughout the majority of the study. Body weight gains in F_0 females at 1500 ppm during pre-pairing, gestation and lactation were generally lower than control values, on occasion achieving statistical significance. Body weight gains of males given 500 or 1500 ppm and females given 500 ppm were similar to control values. Mean F_1 body weight, body weight gain and feed consumption values were slightly lower than those of controls in the group given 1500 ppm. Body weights, body weight gains and feed consumption of animals given 500 ppm were generally similar to control values for both males and females.

The NOAEL in this range-finding study in parents and offspring was 500 ppm (equivalent to 50 mg/kg bw per day), based on lower feed consumption throughout treatment, lower body weight gain that attained statistical significance on occasion and markedly higher liver weights in the F_0 and F_1 generations at 1500 ppm (equivalent to 150 mg/kg bw per day). Occasionally lower feed consumption values at 500 ppm were not considered adverse (Richmond, 2009).

A two-generation study of reproductive toxicity in rats was conducted. Four groups of HanRcc:WIST (SPF) rats (P generation) (25 of each sex per group) received sedaxane (lot no. SMU6LP006; purity 95.3%; ratio of isomers 83.0% *trans* to 12.3% *cis*) in the diet at 0, 200, 500 or 1500 ppm for 10 weeks and were then paired (one male with one female) for mating. The mean compound intakes during the different parts of this study are given in Table 38. The F_1 generation rats were selected from the weaned F_1 litters. The F_1 parents were maintained on test diets for at least 91 days and were then paired for mating. The F_2 offspring were killed at weaning. Dietary sedaxane was administered continuously throughout the study. All dams and remaining pups were killed on day 21 postpartum, and males were killed when they were no longer needed for reproduction.

Homogeneity, stability and achieved concentrations of sedaxane diets were acceptable. All parental animals survived until the scheduled necropsy. Treatment-related findings in clinical observations were not noted at any dose level during the study. In the 1500 ppm group, a reduction in mean feed consumption was noted in the P generation males and in the females in both generations. In F_1 males of the 1500 ppm diet group, body weight and body weight gain were lower throughout the study (Table 39). In females of the 1500 ppm diet group, body weight gain was reduced in the pre-pairing period in both generations and during the lactation period in the P generation, and mean body weight was reduced throughout the study, except on lactation day 14, in both generations.

Table 38. Sedaxane intake of all generations in a multigeneration reproductive toxicity study in rats

	Mean consumption (mg/kg bw per day)		
	200 ppm	500 ppm	1500 ppm
Males P pre-pairing period	16	41	120
Males P after pairing period	11	29	87
Females P pre-pairing period	18	46	143
Females P gestation period	15	40	120
Females P lactation period	36	87	252
Males F_1 pre-pairing period	17	43	134
Males F_1 after pairing period	12	29	89
Females F_1 pre-pairing period	19	47	141
Females F_1 gestation period	16	40	117
Females F_1 lactation period	38	93	282

From Whitlow (2010)

Table 39. Body weights in male rats of P and F_1 generations orally treated with sedaxane

	Body weight (g)							
	P				F_1			
	0 ppm	200 ppm	500 ppm	1500 ppm	0 ppm	200 ppm	500 ppm	1500 ppm
Males								
No. of animals/group	*25*	*25*	*25*	*25*	*25*	*25*	*25*	*25*
Pre-pairing day 1	231	234	231	232	106	105	105	95**
Pre-pairing day 8	267	270	264	267	159	163	162	152
Pre-pairing day 70/71	411	409	405	404	417	414	420	395
Females								
No. of animals/group	*25*	*25*	*25*	*25*	*25*	*25*	*25*	*25*
Pre-pairing day 1	161	161	160	158	96	98	95	89
Pre-pairing day 15	198	201	197	190**	157	158	157	152
Pre-pairing day 70/71	261	263	255	243**	243	241	244	229*
Gestation day 0	259	260	254	240**	254	249	251	237**
Gestation day 21	385	381	380	359**	363	360	365	344
Lactation day 1	279	282	277	263*	273	268	275	253**
Lactation day 7	304	304	301	285**	295	295	298	276**
Lactation day 14	287	299	296	291	289	293	289	284
Lactation day 21	313	314	310	292**	301	300	303	284*

From Whitlow (2010)
* $P < 0.05$; ** $P < 0.01$ (Dunnett test based on pooled variance)

There were no differences in oestrous cyclicity, sperm measurement or reproductive performance between the control and treated animals (Table 40).

Table 40. Reproductive performance in two-generation reproductive toxicity study in rats orally treated with sedaxane

	P generation				F_1 generation			
	0 ppm	200 ppm	500 ppm	1500 ppm	0 ppm	200 ppm	500 ppm	1500 ppm
Number of animals paired	25	25	25	25	25	25	25	25
Mean precoital interval (days)	3.6	3.1	3.2	3.5	4.3	4.0	3.0	5.0
Number of litters	23	25	24	25	23	23	23	25
Mean gestation (days)	21.7	21.6	21.4	21.6	21.7	21.6	21.7	21.6
Percentage mating (%)	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Fertility index (%)	92.0	100.0	96.0	100.0	96.0	92.0	92.0	100.0
Conception rate (%)	92.0	100.0	96.0	100.0	96.0	92.0	92.0	100.0
Gestation index(%)	100.0	100.0	100.0	100.0	95.8	100.0	100.0	100.0

From Whitlow (2010)

Increases in liver weights were observed in males and females of both generations at 1500 ppm (Table 41). In F_1 females, ovary and uterus weights were significantly decreased. Slight but significant changes in organ weights were noted in the adrenal, kidney and thyroid in both sexes in P generation and F_1 females. They were not considered to be treatment related because they were unilateral changes and did not correspond to histopathological changes.

Microscopically, the incidences of centrilobular hepatocellular hypertrophy were increased in males and females of both generations at 1500 ppm (Table 42). The liver hypertrophy corresponding to the increases in liver weights at all treated doses was not considered to be adverse because of the lack of histopathological changes indicating hepatotoxicity. Diffuse follicular cell hypertrophy in the thyroid was slightly increased in F_1 males at 1500 ppm. The increases in vaginal lactation diestrus in F_1 females at 1500 ppm were not considered to be direct effects or hormonal effects of sedaxane on the female reproductive tracts, but rather were considered to reflect a delay in the return to normal cycling due to the lower body weights of the pups that were still suckling at weaning.

In F_1 females at 1500 ppm, ovary and uterus weights were slightly decreased. In morphometric analysis of the ovary at 0 and 1500 ppm, decreases in the numbers of corpora lutea at 1500 ppm in both generations, primordial follicles in the P generation, and growing and antral follicles in the F_1 generation were observed (Table 43). The decrease in primordial follicles in the P generation was not observed in the F_1 generation, which was exposed for a longer time, including the developmental period, the period most sensitive to ovarian toxicants (Hoyer, 2004; Sanbuissho et al., 2009). The decreases in numbers of corpora lutea in the P and F_1 generations might correspond to their litter sizes, which were smaller than those of the controls, but not statistically significantly. Therefore, these decreases in numbers of corpora lutea in the ovary were not considered to be toxicologically significant.

In offspring, there were no effects of sedaxane on litter size, viability or clinical signs. At 1500 ppm, there were no effects on body weights, but there were growth delays 14 days after birth in both sexes of the F_1 and F_2 generations (Table 44).

Although F_1 females at 1500 ppm showed delay in time to vaginal opening, the body weights at sexual maturation were similar to control values, suggesting that the delayed maturation was caused by lower body weights (Table 45). In males in this group, no effect on time until preputial separation was observed. In F_2 pups, anogenital distances in females were slightly, but statistically significantly, increased at 1500 ppm. Although statistically significant, the increase was only slight and was considered to be equivocal. There were no data supporting endocrinological effects on the female reproductive system. Considering the inherent variability in the measurement of anogenital distance

(Tyl, 1999) and the small magnitude of the difference, this was considered not to be toxicologically significant.

Table 41. Selected organ weights in two-generation reproductive toxicity study in rats orally treated with sedaxane

	Mean weight (g)							
	P generation				F_1 generation			
	0 ppm	200 ppm	500 ppm	1500 ppm	0 ppm	200 ppm	500 ppm	1500 ppm
Males								
No. of animals	*25*	*25*	*25*	*25*	*25*	*25*	*25*	*25*
Liver								
- absolute	12.18	12.69	12.93*	14.51**	13.45	13.59	14.23	15.42**
- adjusted	11.995	12.623**	13.086**	14.597**	13.183	13.554	13.962*	15.997**
Females (at the end of lactation)								
No. of animals	*25*	*25*	*25*	*25*	*25*	*25*	*25*	*25*
Liver								
- absolute	13.29	13.35	13.62	15.97**	12.56	13.03	12.73	16.29**
- adjusted[a]	12.937	13.003	13.472	16.786**	12.094	12.713	12.616	17.115**
Ovary (right)								
- absolute	0.056	0.055	0.055	0.049	0.057	0.056	0.054	0.043**
- adjusted[a]	0.055	0.054	0.055	0.052	0.055	0.055	0.054	0.045**
Ovary (left)								
- absolute	0.059	0.056	0.056	0.050*	0.055	0.058	0.054	0.047**
- adjusted[a]	0.058	0.056	0.056	0.051	0.055	0.058	0.054	0.048
Uterus								
- absolute	0.83	0.87	0.96	0.74	0.79	0.80	0.78	0.62**
- adjusted[a]	0.838	0.877	0.964	0.735	0.780	0.791	0.781	0.634*

From Whitlow (2010)

* $P < 0.05$; ** $P < 0.01$ (Dunnett test based on pooled variance)

[a] Organ weight is adjusted for terminal body weight.

Liver weights adjusted for body weight were increased in both sexes of the F_1 and F_1 generations at 1500 ppm.

In this multigeneration reproduction study, the NOAEL for parental toxicity was 500 ppm (equal to 41 mg/kg bw per day), based on lower body weights in both sexes at 1500 ppm (equal to 120 mg/kg bw per day) in P generation males. The NOAEL for reproductive toxicity was 1500 ppm (equal to 120 mg/kg bw per day), the highest dose tested. The NOAEL for offspring toxicity was 500 ppm (equal to 43 mg/kg bw per day), based on lower body weights of F_1 generation males during the pre-pairing period at 1500 ppm (equal to 134 mg/kg bw per day) (Whitlow, 2010).

Table 42. Selected histopathological changes in two-generation reproductive toxicity study in rats orally treated with sedaxane

	Males				Females			
	0 ppm	200 ppm	500 ppm	1500 ppm	0 ppm	200 ppm	500 ppm	1500 ppm
P generation								
Number of animals	*25*	*25*	*25*	*25*	*25*	*25*	*25*	*25*
Hepatocellular hypertrophy	13	6	6	20	0	1	0	10
Thyroid diffuse follicular cell hypertrophy	11	14	11	14	2	0	0	3
Vaginal lactation diestrus	—	—	—	—	7	NE	NE	12
F_1 generation								
Number of animals	*25*	*25*	*25*	*25*	*25*	*25*	*25*	*25*
Hepatocellular hypertrophy	10	7	3	22	0	0	0	17
Thyroid diffuse follicular cell hypertrophy	5	7	3	9	1	0	0	1
Vaginal lactation diestrus	—	—	—	—	8	10	10	20

From Whitlow (2010)
NE, not examined

Table 43. Classification of follicles and the number of corpora lutea in the ovary at the end of lactation in P and F_1 female rats orally treated with sedaxane

Generation	Dose (ppm)	Number of follicles			Number of corpora lutea
		Primordial	Growing	Antral	
P	0	212.10	18.70	8.60	16.10
	1500	139.70*	18.80	7.80	12.20*
F_1	0	218.00	24.50	10.50	15.50
	1500	201.50	13.00*	5.00*	11.00*

From Whitlow (2010)
* $P < 0.05$ (Wilcoxon test)

Table 44. Body weights of F_1 and F_2 pups in rats orally treated with sedaxane

	Males				Females			
	0 ppm	200 ppm	500 ppm	1500 ppm	0 ppm	200 ppm	500 ppm	1500 ppm
F_1 generation								
Number of litters/group	23	25	24	25	23	25	24	25
Mean pup body weight (g)								
- day 1	6.3	6.2	6.1	6.3	6.0	6.0	5.7	6.0
- day 4	8.9	9.1	8.6	9.3	8.6	8.8	8.3	8.9
- day 7	15.0	15.1	14.4	14.3	14.3	14.6	13.9	13.9

Table 44 (continued)

	Males				Females			
	0 ppm	200 ppm	500 ppm	1500 ppm	0 ppm	200 ppm	500 ppm	1500 ppm
- day 14	30.7	31.3	29.8	28.4**	29.6	30.5	29.4	27.7*
- day 21	50.0	49.9	48.2	45.1**	47.8	48.5	46.9	43.3**
F_2 generation								
Number of litters/group	23	23	23	25	23	23	23	25
Mean pup body weight (g)								
- day 1	6.5	6.5	6.5	6.5	6.2	6.1	6.1	6.1
- day 4	9.7	9.7	9.4	9.5	9.4	9.3	9.0	9.1
- day 7	22.7	22.6	21.8	21.5	22.0	21.9	21.3	21.1
- day 14	31.8	32.0	31.0	29.9*	31.1	31.1	30.2	29.3*
- day 21	51.4	51.6	50.1	46.9**	49.6	49.5	48.0	45.7**

From Whitlow (2010)
* $P < 0.05$; ** $P < 0.01$ (Dunnett test based on pooled variance)

Table 45. Selected sexual developmental landmarks (preputial separation, vaginal opening and anogenital distance) in F_1 and F_2 offspring in rats orally treated with sedaxane

	Males				Females			
	0 ppm	200 ppm	500 ppm	1500 ppm	0 ppm	200 ppm	500 ppm	1500 ppm
F_1 generation								
Number of animals	25	25	25	25	25	25	25	25
Day of preputial separation/vaginal opening	27.6	27.9	28.0	28.1	32.5	33.3	32.7	34.2**
Weight at preputial separation/vaginal opening (g)	82.98	83.64	81.96	75.58**	99.59	104.52	100.70	102.58
F_2 generation								
Number of litters	23	23	23	25	22	23	23	25
Anogenital distance (mm)	3.63	3.66	3.80	3.73	1.79	1.85	1.88	1.93*

From Whitlow (2010)
* $P < 0.05$; ** $P < 0.01$ (Dunnett test based on pooled variance)

(b) Developmental toxicity

Rats

A dose range–finding developmental toxicity study was performed in which groups of 10 mated female Han Wistar rats were dosed orally by gavage with sedaxane (lot no. SMU6LP006; purity 95.3%; ratio of isomers 83.0% *trans* to 12.3% *cis*) at 0, 200, 500 or 750 mg/kg bw per day on days 6–20 post-coitum using 0.5% carboxymethylcellulose as the vehicle. Dams were examined for mortality or morbidity twice daily. Clinical signs were assessed and recorded daily. Individual weights were recorded daily. Feed consumption was recorded on days 0–4, 4–6, 6–9, 9–12, 12–15, 15–18 and 18–21 post-coitum. All surviving dams were killed on day 21 post-coitum, and the fetuses were

removed by caesarean section. Examination of dams and external and visceral examinations of all fetuses were performed.

The dams in the groups receiving 500 and 750 mg/kg bw per day were killed before the scheduled end of the study because of their poor condition, consisting of decreased activity, generally poor clinical condition and reduced feed consumption and body weight gains. Feed consumption, body weight gain and corrected body weight gain were statistically significantly reduced at 200 mg/kg bw per day. No sedaxane-related effects were noted in the reproduction or fetal data at this dose level. Based on the results of this study, sedaxane dose levels of up to 200 mg/kg bw per day delivered orally by gavage were considered suitable for a main developmental toxicity study in rats (Whitlow, 2009).

In a developmental toxicity study, groups of 24 mated female Han Wistar rats were dosed orally by gavage with sedaxane (lot no. SMU6LP006; purity 95.3%; ratio of isomers 83.0% *trans* to 12.3% *cis*) at 0, 25, 100 or 200 mg/kg bw per day on days 6–20 post-coitum using 0.5% carboxymethylcellulose as the vehicle. Dams were examined for mortality or morbidity twice daily. Clinical signs were assessed and recorded daily. Individual body weights were recorded daily. Feed consumption was recorded on days 0–4, 4–6, 6–9, 9–12, 12–15, 15–18 and 18–21 post-coitum. All surviving dams were killed on day 21 post-coitum, and the fetuses were removed by caesarean section. External examinations of all dams and fetuses were conducted. In addition, external examination of all fetuses and visceral or skeletal examination of approximately equal numbers of fetuses from each treatment group were conducted.

At 200 mg/kg bw per day, mean feed consumption and mean body weights were reduced during the treatment period in dams. At 100 mg/kg bw per day, feed consumption and cumulative body weight gain of dams were slightly, but significantly, reduced during the 1st and 2nd weeks of gestation. A slight reduction in fetal weight was noted only in females at 200 mg/kg bw per day (4.5 g) compared with controls (4.7 g). This reduction in fetal body weights in female pups was not considered to be adverse because it represented a difference of only 4.3% compared with controls, this effect was not observed in male pups and there were no differences in the combined (both sexes) fetal body weights. In addition, no effects were observed on fetal development, and there was no delay in the stage of ossification observed. No sedaxane-related effects on fetal survival or the numbers and types of fetal abnormalities and variations were noted in any group.

In this developmental toxicity study in rats, the NOAEL for maternal toxicity was 25 mg/kg bw per day, based on reduction of feed consumption and body weight gain at 100 mg/kg bw per day. The NOAEL for developmental toxicity was 200 mg/kg bw per day, the highest dose tested. Sedaxane was not teratogenic (Cappon et al., 2005; Senn, 2009).

Rabbits

A dose range–finding developmental toxicity study was performed in which groups of 10 time-mated female New Zealand White (Hra:(NZW)SPF) rabbits 6 months of age were dosed orally by gavage with sedaxane (lot no. SMU6LP006; purity 95.3%; ratio of isomers 83.0% *trans* to 12.3% *cis*) at 0, 100, 300 or 500 mg/kg bw per day on days 7–28 post-coitum using 0.5% carboxymethylcellulose as the vehicle. Mortality, clinical observations, body weights and feed consumption were recorded. On gestation day 29, the uteri, placentae and ovaries of all surviving dams were examined, and the numbers of fetuses, early and late resorptions, total implantations and corpora lutea were recorded. Gravid uterine weights and liver weights from all females were recorded. The fetuses were weighed, sexed and examined for external and visceral malformations and developmental variations.

Severe toxicity observed in two dams of the 500 mg/kg bw per day group led to their euthanasia on gestation day 16. A dose-related increase in the incidence of decreased defecation was noted among surviving rabbits in all treated groups. Body weight losses were noted at 300 and 500 mg/kg bw per day immediately following administration of sedaxane on gestation days 7–10, and

lower body weight gains continued at 500 mg/kg bw per day during gestation days 10–13. Reductions in feed consumption at 300 and 500 mg/kg bw per day were continued during gestation days 13–21, but without corresponding effects on body weight gains and when the overall treatment period was evaluated. Body weights were depressed throughout the treatment period at 300 and 500 mg/kg bw per day by up to 8.9%. No treatment-related changes were observed at necropsy. Dose-related higher absolute and body weight–relative liver weights were noted at all dose levels, the increases being statistically significant at 300 and 500 mg/kg bw per day. There was no evidence of developmental toxicity at any dose level. No external or visceral fetal malformations or developmental variations were attributable to sedaxane administration to the dams.

On the basis of these results, dose levels of 25, 100 and 200 mg/kg bw per day were selected for a definitive prenatal developmental toxicity study of sedaxane in New Zealand White rabbits (Sawhney Coder, 2010a).

In the definitive developmental toxicity study, groups of 25 time-mated female New Zealand White (Hra:(NZW)SPF) rabbits approximately 6 months of age were dosed orally by gavage with sedaxane (lot no. SMU6LP006; purity 95.3%; ratio of isomers 83.0% *trans* to 12.3% *cis*) at a dose of 0, 25, 100 or 200 mg/kg bw per day on days 7–28 post-coitum, using 0.5% carboxymethylcellulose as the vehicle. All rabbits were observed twice daily for mortality and morbidity. Clinical observations, body weights and feed consumption were recorded. On gestation day 29, the uteri, placentae and ovaries from all surviving dams were examined, and the numbers of fetuses, early and late resorptions, total implantations and corpora lutea were recorded. Gravid uterine weights and net body weight (the gestation day 29 body weights exclusive of the weight of the uterus and contents), net body weight change (the gestation days 0–29 body weight change exclusive of the weight of the uterus and contents) and cumulative body weight were recorded. In addition, the livers from all females examined at the scheduled necropsy were weighed. The fetuses were weighed, sexed and examined for external, visceral and skeletal malformations and developmental variations.

At 200 mg/kg bw per day, decreased maternal body weight gain, cumulative body weight gain and feed consumption were observed during treatment (Table 46). Slight increases in liver weights, both absolute weight (13%) and adjusted to net body weight as a covariate (14%), were observed at 200 mg/kg bw per day when compared with controls. Liver weight increases of 11% (absolute) and 9% (adjusted) observed at 100 mg/kg bw per day were considered adaptive.

Fetal examination revealed slightly lower mean fetal weights at 200 mg/kg bw per day, although there were no treatment-related effects on fetal development in this group.

In the developmental toxicity study in rabbits, the NOAEL for maternal toxicity was 100 mg/kg bw per day, based on the reduction of body weight gain and feed consumption at 200 mg/kg bw per day. The NOAEL for developmental toxicity was 100 mg/kg bw per day, based on slight reduction of fetal body weights at 200 mg/kg bw per day (Sawhney Coder, 2010b).

2.6 *Special studies*

(a) Acute neurotoxicity

In a preliminary acute oral neurotoxicity study in rats, groups of three male and three female HanRcc:Wistar (SPF) rats were administered a single oral dose of sedaxane (lot no. SMU6LP006; purity 95.3%; ratio of isomers 83.0% *trans* to 12.3% *cis*) at 0, 80, 1000 or 2000 mg/kg bw and observed for 8 days.

All animals survived. At 1 hour after dosing, one female exhibited slight inactivity at 2000 mg/kg bw. Treatment-related effects at 1000 and 2000 mg/kg bw were a transient occurrence of reduced activity, recumbency, hunched posture, spasms, ruffled fur, piloelection and uncoordinated movements, with a peak effect observed on day 1 in males and females at 3 hours after the dosing. Sedaxane did not affect body weight or macroscopic findings.

Table 46. Body weight and cumulative body weight gain in pregnant rabbits orally treated with sedaxane

	Body weight / body weight gain (g)			
	0 mg/kg bw per day (control)	25 mg/kg bw per day	100 mg/kg bw per day	200 mg/kg bw per day
Body weight				
- GD 0	3348	3346	3355	3365
- GD 7	3451	3511	3479	3462
- GD 8	3440	3508	3460	3425
- GD 14	3507	3606	3550	3455
- GD 19	3539	3687	3590	3484
- GD 24	3588	3747	3637	3557
- GD 29	3602	3711	3643	3549
Body weight gain				
- GD 7	0	0	0	0
- GD 8	−11	−3	−19	−37**
- GD 9	−10	−3	−7	−35*
- GD 11	4	20	3	−30
- GD 14	55	95	71	−7*
- GD 18	89	161	99	10
- GD 20	97	192*	122	36
- GD 23	126	235*	163	73
- GD 29	150	196	159	71

From Sawhney Coder (2010b)

GD, gestation day; * $P < 0.05$; ** $P < 0.01$

Based on the results, it was determined that a single oral sedaxane dose of 2000 mg/kg bw would be an appropriate high dose for an acute neurotoxicity study in rats (Sommer, 2008).

To detect acute oral neurotoxicity in rats, four groups of 10 male and 10 female HanRcc:Wistar (SPF) rats were administered a single oral dose of sedaxane (lot no. SMU6LP006; purity 95.3%; ratio of isomers 83.0% *trans* to 12.3% *cis*) at 0 (0.5% carboxymethylcellulose, the vehicle control), 30, 250 or 2000 mg/kg bw per day, respectively, and observed for 15 days. General cage-side observations, detailed clinical observations comprising open-field evaluation of clinical signs, functional observational battery evaluations and locomotor activity were assessed. Feed consumption and body weights were measured during the test period. On day 16, five rats of each sex per group were perfusion fixed in situ, and selected nervous system tissues were collected, processed and examined microscopically.

Four males and three females in the 2000 mg/kg bw group were killed due to the severity of the clinical signs in response to sedaxane on day 1. Clinical signs observed in these rats included markedly laboured respiration (bradypnoea), abnormal gait, moderately reduced activity, weakened condition, recumbency, piloerection and/or a decrease in rectal temperature. No remarkable macroscopic findings were noted in these rats at necropsy. The remaining rats survived until the end of the scheduled post-dosing observation period.

At approximately 1 hour after dosing, treatment-related cage-side observations for the 250 and 2000 mg/kg bw females included weakened condition, swaying gait and decreased activity. At approximately 2 hours after dosing, treatment-related clinical observations noted during the functional

observational battery included increased incidences of reduced activity, decreased rearing, initial inactivity, piloerection (250 and 2000 mg/kg bw males and females), reduced muscle tone (250 mg/kg bw females and 2000 mg/kg bw males and females), hunched posture (2000 mg/kg bw males), recumbency (250 and 2000 mg/kg bw males), abnormal gait (2000 mg/kg bw females) and bradypnoea (2000 mg/kg bw males and females). Similar signs and incidences were noted when detailed observations were made approximately 5 hours after dosing. Other treatment-related findings noted in the functional observational battery evaluations at approximately 2 hours after dosing included lower mean body temperatures and decreases in both mean forelimb and hindlimb grip strength for the 2000 mg/kg bw males and females. At approximately 3–4 hours after dosing, the locomotor parameters for total moved distance and rearing activity were reduced at 250 and 2000 mg/kg bw in males and females. The decreases in these locomotor activity parameters are attributed to treatment and correlate with the increased incidences of reduced activity and decreased rearing activity observed in the detailed observations approximately 2–3 hours after dosing. During days 2–7, several treatment-related daily clinical signs were noted for the 250 and 2000 mg/kg bw males and females and included ruffled fur (250 mg/kg bw males and 2000 mg/kg bw males and females), rough coat (2000 mg/kg bw males), weakened condition (250 mg/kg bw females and 2000 mg/kg bw males and females), swaying gait (250 and 2000 mg/kg bw females), decreased activity and prostrate and hunched posture (2000 mg/kg bw females). Treatment with doses of 250 and 2000 mg/kg bw resulted in a dose-dependent decrease in mean feed consumption for males and females on days 1–2. Lower mean body weights for the 250 mg/kg bw males and 2000 mg/kg bw males and females were observed on day 8, and these reflected the lower mean body weight gains noted in these groups during the 1st week post-dosing. No treatment-related cage-side and/or detailed clinical observations were noted subsequent to the 1st week of the study (i.e. days 8–16). There were no effects on brain weight or macroscopic or microscopic findings. At study termination, no neurohistopathology was observed in any treated groups.

The NOAEL for systemic toxicity following a single oral dose was 30 mg/kg bw, based on severe loss of general condition, decreased body weight and decreased feed consumption at 250 mg/kg bw in both males and females (Sommer, 2009b).

(b) Subchronic neurotoxicity

A 28-day dietary toxicity study was conducted as a preliminary range-finder to a 90-day neurotoxicity study using groups of eight male and eight female Wistar (HanRcc:WIST) rats orally treated with sedaxane (lot no. SMU6LP006; purity 95.3%; ratio of isomers 83.0% *trans* to 12.3% *cis*) at 0 (control), 500, 2000 or 5000 ppm (equal to 0, 37.8, 153.5 and 360.1 mg/kg bw per day for males and 0, 40.1, 156.4 and 338.8 mg/kg bw per day for females, respectively). All animals were checked for clinical condition and detailed behavioural observations. Body weights and feed consumption were recorded. At the end of the scheduled period, animals were necropsied.

All animals survived their scheduled study period. No treatment-related clinical signs were observed in any rats of any dose groups throughout the study period. Reduced feed intake was observed in males and females at 5000 ppm and females at 2000 ppm. Feed utilization was significantly reduced in males and females at 5000 ppm. Body weights and cumulative body weight gains were reduced in males and females at 5000 ppm and in females at 2000 ppm.

The lower body weights (−10.0% in males, −16.5% in females) and cumulative body weight gain (−27.1% in males, −52.8% in females) at 5000 ppm over 4 weeks indicated that 5000 ppm was too high to be the top dose in the subchronic neurotoxicity study. Consistently lower feed consumption and feed utilization also confirmed the observation concerning the 5000 ppm dose level. No macroscopic findings were recorded at necropsy in any animals of any dose groups (Sommer, 2009c).

Groups of 12 male and 12 female HanRcc:WIST (SPF) rats were fed sedaxane (lot no. SMU6LP006; purity 95.3%; ratio of isomers 83.0% *trans* to 12.3% *cis*) in the diet at a concentration of 0, 300, 1000 or 4000 ppm (equal to 0, 19.7, 66.0 and 260.0 mg/kg bw per day for males and 0,

24.3, 79.7 and 302.9 mg/kg bw per day for females, respectively) for up to 92 days. All animals were observed prior to the study start and daily throughout the study for any changes in clinical condition, detailed clinical observations, a functional observational battery and an assessment of locomotor activity. Body weight and feed consumption were measured. Ophthalmoscopic examination was performed in all animals. At the end of the scheduled period, five rats of each sex per group were perfused in situ, brain weight was recorded, and organs and selected nervous tissues were assessed for macroscopic changes. The remaining animals were killed and discarded. Tissues of the peripheral and central nervous systems of the control and 4000 ppm dose groups were examined neurohistopathologically.

Homogeneity, stability and achieved concentration of sedaxane diets were acceptable. All animals survived the scheduled study period. Detailed clinical observations and functional observational battery evaluations of clinical symptoms revealed no treatment-related effects. Body temperature, landing foot splay and grip strength in the forelimbs and hindlimbs were not affected. Feed consumption was significantly reduced in both sexes at 4000 ppm. At 4000 ppm, body weights were generally lower in both sexes throughout the study (Table 47). Cumulative body weight gains were statistically significantly lower in both sexes at 4000 ppm from day 8 to the end of study. Mean locomotor activity total distance values in both sexes at 4000 ppm were generally lower than in controls. These lower total distance values are not considered to represent a direct neurotoxic insult, but rather are reflective of the treatment-related decreases in feed consumption and body weight. No gross findings were present at necropsy. Brain weights and adjusted brain weights were not affected by treatment with sedaxane. There was no treatment-related neuropathology.

Table 47. Body weight changes and feed consumption in rats orally treated with sedaxane in a 90-day neurotoxicity study

	Males				Females			
	0 ppm	300 ppm	1000 ppm	4000 ppm	0 ppm	300 ppm	1000 ppm	4000 ppm
Body weight (g)								
- day 1	188.4	194.0	195.3	191.5	150.9	147.7	146.6	151.2
- day 8	233.6	241.1	243.1	225.8	173.1	171.7	168.2	164.7
- day 15	268.1	277.4	279.2	258.6	188.2	185.7	182.6	174.8**
- day 29	320.7	331.3	332.3	299.6	218.6	213.7	213.7	202.2*
- day 57	383.1	398.0	397.7	350.9*	243.2	248.0	238.8	225.3
- day 85	426.9	438.8	441.4	386.8*	254.7	258.1	248.9	229.3**
Feed consumption (g/day)								
- week 1	19.925	19.670	20.071	14.645**	15.770	15.749	15.560	10.280**
- week 7	23.325	24.008	23.093	21.055*	17.135	18.586	17.519	15.650
- week 8	22.761	23.289	23.403	20.712*	19.457	18.985	18.210	15.016**
- week 9	23.135	23.221	23.403	20.917*	15.931	17.978	16.866	15.143
- week 10	22.758	22.903	23.029	20.815	17.359	17.278	16.641	14.805**
- week 11	22.877	22.870	23.687	21.465	17.258	19.200	17.802	14.855*

From Sommer (2009d)

* $P < 0.05$; ** $P < 0.01$ (Dunnett's *t*-test, two-sided)

The NOAEL for systemic toxicity in a 13-week neurotoxicity study in rats was 1000 ppm (equal to 66 mg/kg bw per day), based on decreased body weight, body weight gain, feed consumption and feed efficiency, as well as reduced locomotor activity, at 4000 ppm (equal to 260 mg/kg bw per day). Sedaxane has no neurotoxicity (Sommer, 2009d).

(c) Immunotoxicity

To provide information on suppression of the immune system that might occur as a result of repeated exposure to a test chemical, the effects of oral (dietary, ad libitum) treatment with sedaxane (lot no. SMU6LP006; purity 95.3%; ratio of isomers 83.0% *trans* to 12.3% *cis*) on the humoral component of the immune system were investigated in male CD-1 mice. The following immunological parameters were evaluated: spleen weights, thymus weights, spleen cell number and the spleen immunoglobulin M (IgM) antibody response to the T cell–dependent antigen, sheep erythrocytes (sRBC). Four groups of male Crl:CD1 (ICR) mice were fed sedaxane ad libitum in the diet for a minimum of 28 consecutive days at 0, 500, 2000 or 5500 ppm (equal to 0, 93, 637 and 1084 mg/kg bw per day, respectively). Five mice were administered the positive control substance, cyclophosphamide monohydrate, via intraperitoneal injection (50 mg/kg bw per day) for 4 consecutive days (study days 24 through 27). Additionally, all mice were immunized with an intravenous injection of sRBC on study day 24, approximately 96 hours prior to the scheduled necropsy. Each group consisted of 10 males. All animals were euthanized on study day 28. Spleen samples were processed into single-cell suspensions, and an antibody-forming cell (AFC) assay was performed to determine the number of specific IgM antibody-forming cells directed towards sRBC. The AFC assay was a modification of the Jerne plaque assay (Jerne, Nordin & Henry, 1963).

Homogeneity, stability and achieved concentration of sedaxane diets were acceptable. All animals survived to the scheduled necropsy. There were no test substance–related clinical observations, body weights, feed consumption, macroscopic findings, terminal body weights or absolute, adjusted or relative spleen or thymus weights. There were no effects on the mean number of IgM antibody-forming splenocytes that were attributed to sedaxane.

Adjusted liver weights were increased at 5500 ppm, but this finding was considered non-adverse due to the lack of histopathology indicating hepatotoxicity. In the cyclophosphamide group, there were findings consistent with the known immunosuppressant effects (Table 48). In the sedaxane-treated groups, there was no suppression of the humoral immune component of the immune system, including a lack of changes in spleen and thymus weights, spleen cell numbers and the T cell–dependent antibody response of splenocytes, also referred to as the IgM AFC assay.

Table 48. Selected organ weights in an immunotoxicity study in mice orally treated with sedaxane for 28 days

Parameter	Mean values				
	0 ppm	500 ppm	2000 ppm	5500 ppm	Positive control CP: 50 mg/kg bw
Terminal body weight (g)	35.1	33.9	35.2	34.4	34.3
Absolute liver weight (g)	2.1248	2.0143	2.1504	2.2802	2.0587
Adjusted liver weight (g)	2.0858	2.0669	2.1034	2.2930**	2.0794
Absolute thymus weight (g)	0.0377	0.0379	0.0387	0.0428	0.0122**
Adjusted thymus weight (g)	0.0377	0.0378	0.0387	0.0428	0.0122**
Relative thymus weight (% of body weight)	0.11	0.11	0.11	0.12	0.04**
Absolute spleen weight (g)	0.1358	0.1271	0.1355	0.1469	0.0629**
Adjusted spleen weight (g)	0.1338	0.1297	0.1331	0.1476	0.0640**
Relative spleen weight (% of body weight)	0.38	0.38	0.39	0.43	0.19**

From Crittenden (2010)

CP, cyclophosphamide monohydrate; ** $P < 0.01$ (Dunnett's test, two-sided)

In this immumotoxicity study in mice, sedaxane was not immunotoxic at doses up to 5500 ppm (equal to 1084 mg/kg bw per day) (Crittenden, 2010).

(d) Comparative toxicities of trans *and* cis *isomers and their mixture*

To investigate the relative toxicity profiles of the *trans* isomer, the *cis* isomer and a 1:1 mix of these isomers (lot no. KI 7193/5 for *trans* isomer and lot no. KI-7245/5 for *cis* isomer; purity not confirmed), an exploratory investigation of 28-day dietary toxicity in rats, with supplemental examination of liver biochemistry, toxicokinetic and serum thyroid hormone parameters, was conducted.

Groups of five male and five female HsdBrlHan:Wistar rats were fed diets containing 0, 500, 2000 or 5000 ppm of the *trans* or *cis* isomers or a 1:1 mixture of the two isomers for 28 consecutive days (see Table 49 for mean intakes). Clinical observations, body weights and feed consumption were measured throughout the study. At the end of the exposure period, the rats were killed and examined post mortem. Cardiac blood samples were taken for clinical chemistry and analysis of thyroid hormones, selected organs were weighed and specified tissues were taken for subsequent microscopic examination. Samples of liver were analysed for total cytochrome P450 (CYP) and CYP-dependent isoenzyme activities. Satellite groups of three male and three female rats per sampling day (exposure days 1 and 14) were treated with the same test diets and utilized for toxicokinetic analysis. Blood samples for toxicokinetic analysis were taken 8 hours after the administration of test diets and at 4-hour intervals thereafter (approximately at 17:00, 21:00, 01:00, 05:00, 09:00 and 13:00) on day 1/2 and on day 14/15 of the study.

Table 49. Mean chemical intakes

	Mean intake (mg/kg bw per day)								
	1:1 mixed isomers			*Trans* isomer			*Cis* isomer		
	500 ppm	2000 ppm	5000 ppm	500 ppm	2000 ppm	5000 ppm	500 ppm	2000 ppm	5000 ppm
Males	47.5	181.2	444.6	47.0	187.4	438.2	45.9	182.7	438.2
Females	46.7	181.1	428.1	48.4	177.1	384.3	47.6	179.6	435.8

From Peffer & Noakes (2010)

There were no deaths or treatment-related clinical signs. Body weights adjusted for initial weights were lower, compared with the controls, for males and females that received the *trans* or *cis* isomer or mixed isomers at 5000 ppm (Table 50). The magnitude of the difference from the controls was consistent across all three test compounds. There was a slightly lower body weight, compared with the controls, for males and females at 2000 ppm, although this was generally not of statistical significance.

Feed consumption was lower than that of the controls for both males and females that received the *trans* isomer, *cis* isomer or mixed isomers at 5000 ppm and to a lesser extent for females at 2000 ppm. In the toxicokinetics analysis, there were no clear differences in mean T_{max} between the *trans* isomer, *cis* isomer and mixed isomers. The data were limited (no available data to determine the overall absorption and rate of conversion to metabolites for the *trans* and *cis* isomers); however, mean C_{max} and AUC were generally considerably lower (approximately 5- to 20-fold, depending on sex, sampling day, dietary concentration and parameter) for the *cis* isomer compared with the *trans* isomer when administered separately or when administered as mixed isomers with *trans* and *cis* isomers analysed separately in the plasma (Table 51).

Table 50. Body weights of rats orally treated with* trans, cis *and mixed isomers of sedaxane

Day	Body weight[a] (g)							
	Males				Females			
	0 ppm	500 ppm	2000 ppm	5000 ppm	0 ppm	500 ppm	2000 ppm	5000 ppm
trans isomer								
- day 1	158.0	156.6	158.4	157.4	124.8	123.0	119.8	123.4
- day 7	193.4	193.8	193.0	174.1**	137.9	141.1	138.4	124.9**
- day 14	227.9	230.9	225.5	203.1**	154.4	158.1	151.5	133.9**
- day 21	253.9	254.0	254.3	221.0**	166.5	170.6	162.8	143.8**
- day 29	280.7	284.5	284.5	238.4**	180.5	183.9	171.2	149.3**
cis isomer								
- day 1	158.0	158.0	157.8	158.6	124.8	123.2	121.2	127.8
- day 7	193.4	194.8	188.5	172.8**	137.9	139.1	138.7	123.8**
- day 14	227.9	228.9	221.9	194.2	154.4	156.7	153.0	135.4**
- day 21	253.9	256.1	240.1	212.3**	166.5	168.6	164.6	148.0**
- day 29	280.7	281.5	260.3*	231.1**	180.5	182.5	170.9	155.4**
Mixture								
- day 1	158.0	159.6	156.2	159.8	124.8	122.0	120.0	117.2
- day 7	193.4	194.5	190.6	169.1**	137.9	144.2	133.2	133.9
- day 14	227.9	234.5	223.7	196.4**	154.4	160.8	153.7	142.7**
- day 21	253.9	264.0	245.1	217.2**	166.5	168.2	164.8	152.5**
- day 29	280.7	294.7	268.0	236.1**	180.5	183.0	176.6	160.5**

From Peffer & Noakes (2010)

* $P < 0.05$; ** $P < 0.01$ (Student's *t*-test, two-sided)

[a] Values are the mean body weights adjusted for initial body weights by analysis of covariance.

Haematology parameters were unaffected, with the exception of a slightly higher, but not dose-related, prothrombin time in males treated with the *trans* isomer at 2000 and 5000 ppm.

In blood chemistry investigations, increases in plasma total protein, cholesterol and triglyceride concentrations were common changes in both sexes receiving the *trans* isomer, *cis* isomer or mixed isomers at higher doses (Table 52); triglyceride concentrations were increased in females in all groups treated with the *trans* isomer and mixed isomers, and cholesterol levels were increased in females in all groups treated with the *cis* isomer. Slight increases in alkaline phosphatase, gamma-glutamyl transferase or alanine aminotransferase were also noted in males or females dosed with the three compounds at 5000 ppm.

None of the three test compounds caused an effect on serum thyroid hormone levels (triiodothyronine, thyroxine or thyroid stimulating hormone).

Analyses of hepatic CYP-related enzymes showed that pentoxyresorufin *O*-dealkylase (PROD) activity was increased in males only when receiving the *trans* isomer at 500 ppm, in both males and females receiving the *cis* isomer or the mixed isomers at 500 ppm and in both males and females receiving all three test substances at 2000 or 5000 ppm. Increases were greater in males (up to about 40-fold) than in females (up to about 10-fold). The marked increase in hepatic PROD activity with all three test compounds indicates that they are potent inducers of CYP2B isoforms. Analysis of ethoxyresorufin *O*-dealkylase (EROD) activity showed that, in male rats, the *cis* isomer apparently caused increases in activity in the 500 and 2000 ppm diet groups of 2-fold and 1.6-fold, respectively,

but there was no increase in activity in the 5000 ppm diet group, suggesting either that the higher activities observed in the lower dose groups were not an effect of treatment or that it was the lower activity in the highest dose level group that was the statistical anomaly. In female rats, EROD activity (per milligram protein) was slightly increased by the *cis* isomer in all dose groups by about 1.5-fold. The *trans* isomer had no clear effect on the activity of EROD in male rats, and in females, there was only a slight increase in activity in the 5000 ppm *trans* isomer diet group. The diet containing the mixture of isomers was associated with higher EROD activity in male rats of the 2000 ppm diet group and in females of the 2000 and 5000 ppm diet groups. In both sexes, the increases were modest (1.5- to 1.7-fold in specific activity). The weakly increased EROD activity indicates that the three different test compounds possess low potential for inducing CYP1A isoforms. There were no consistent effects of treatment on total CYP in the liver.

Table 51. Toxicokinetics analysis of* trans*,* cis *and mixed isomers in rats orally treated with sedaxane for 28 days

Dietary concentration (ppm)	Sex	Day	*Trans*			*Cis*		
			Mean T_{max} (h)	Mean C_{max} (µg/ml)	Mean AUC (µg·h/ml)	Mean T_{max} (h)	Mean C_{max} (µg/ml)	Mean AUC (µg·h/ml)
500	Male	1	12	0.391	4.89	—	—	—
		14	13	0.202	1.85	—	—	—
	Female	1	13	0.198	2.73	—	—	—
		14	17	0.222	2.74	—	—	—
2000	Male	1	16	4.61	59.0	20	0.676	5.53
		14	16	0.568	6.18	20	0.315	3.15
	Female	1	13	2.50	28.6	16	0.266	2.63
		14	17	2.43	25.0	15 (18)[a]	0.681 (0.265)	6.01 (2.46)
5000	Male	1	19	3.40	44.8	16	0.526	6.45
		14	19	4.75	83.8	18	0.196	2.17
	Female	1	20	3.37	44.6	16	0.635	6.38
		14	17	5.11	81.7	17	1.50	10.0
Mixture (*trans*:*cis* 1:1)								
500	Male	1	17	0.112	1.42	20	0.0275	0.320
		14	12	0.0603	0.633	8	0.0319	0.231
	Female	1	12	0.0915	1.23	—	—	—
		14	16	0.145	1.51	—	—	—
2000	Male	1	19	1.22	14.3	19	0.101	1.42
		14	19	0.153	2.16	—	—	—
	Female	1	16	0.555	8.48	17	0.134	1.50
		14	19	0.471	5.34	20	0.0412	0.348
5000	Male	1	23	1.61	21.2	19	0.120	2.03
		14	17	0.613	6.15	18	0.0318	0.328
	Female	1	19	0.843	11.4	19	0.147	1.78
		14	20	1.290	14.1	20	0.0644	0.926

From Peffer & Noakes (2010)
—, data not available; AUC, area under the plasma–concentration time curve; C_{max}, peak plasma concentration; T_{max}, time to reach C_{max}
[a] Standard deviation given in parentheses.

Table 52. Summary of comparison of clinical chemistry in a 4-week oral study in rats treated with* trans *or* cis *isomers or their mixture

	Males				Females			
	0 ppm[a]	500 ppm	2000 ppm	5000 ppm	0 ppm[a]	500 ppm	2000 ppm	5000 ppm
***Trans* isomer**								
Total protein (g/l)	58.3	57.9	58.8	64.5**	59.3	62.5	64.4*	66.7**
Total bilirubin (µmol/l)	4.79	4.06	3.97	10.36**	4.00	5.42	4.38	7.92*
Cholesterol (mmol/l)	1.63	1.67	1.39	2.66**	1.50	1.70	2.34**	3.61**
Triglycerides (mmol/l)	1.26	1.42	1.59	5.29**	0.95	1.75*	2.46**	4.91**
Alkaline phosphatase (IU/l)	398	395	322*	495*	225	224	220	264
Gamma-glutamyl transferase (IU/l)	6.8	7.0	7.3	6.8	5.5	5.5	6.8	10.2**
Alanine aminotransferase (IU/l)	67.4	65.6	61.4	74.6	44.0	42.0	53.0	62.6*
***Cis* isomer**								
Total protein (g/l)	58.3	59.5	62.7*	63.1*	59.3	63.3*	62.9	65.2*
Total bilirubin (µmol/l)	4.79	3.19	3.85	5.76	4.00	4.10	4.31	5.48
Cholesterol (mmol/l)	1.63	1.92	1.94	2.32**	1.50	1.83*	2.07**	2.90**
Triglycerides (mmol/l)	1.26	1.34	1.77	2.41*	0.95	1.32	2.38**	2.76**
Alkaline phosphatase (IU/l)	398	363	348	421	225	221	259	230
Gamma-glutamyl transferase (IU/l)	6.8	7.1	8.2	13.4**	5.5	5.9	7.0	11.7**
Alanine aminotransferase (IU/l)	67.4	64.8	49.6	54.8	44.0	58.2	46.2	51.8
Mixture (*trans:cis*, 1:1)								
Total protein (g/l)	58.3	59.2	59.8	66.7**	59.3	62.9	63.5	64.7**
Total bilirubin (µmol/l)	.4.79	4.09	4.59	6.71	4.00	5.35	3.35	10.28**
Cholesterol (mmol/l)	1.63	1.64	1.67	2.83**	1.50	1.65	2.15**	2.94**
Triglycerides (mmol/l)	1.26	1.66	1.69	3.36**	0.95	2.04*	1.77*	3.14**
Alkaline phosphatase (IU/l)	398	458	375	477*	225	228	198	246
Gamma-glutamyl transferase (IU/l)	6.8	6.7	6.8	12.1**	5.5	6.0	7.6	8.2*
Alanine aminotransferase (IU/l)	67.4	67.2	63.8	61.2	44.0	44.2	45.3	64.2*

From Peffer & Noakes (2010)
IU, international unit; * $P < 0.05$; ** $P < 0.01$
[a] Compared with satellite control group.

All three compounds significantly increased the 16β-hydroxylation of testosterone in both males (36-fold) and females (20-fold), consistent with being potent inducers of CYP2B isoforms. In addition, the three test compounds caused decreased 16α- and 2α-testosterone hydroxylase activities in male rats but increased their activities in female rats, and there was a greater increase in the 2β- and 6β-hydroxytestosterone hormonal levels in female rats than in males. Immunoblotting showed increased levels of CYP2B and CYP3A relative to the controls for all three compounds, providing further qualitative support for the increased enzyme activity, noted in liver biochemistry, of these two CYP isoenzymes.

There were statistically significantly higher liver weights and liver weights adjusted for body weights for males and females at 2000 and 5000 ppm for all three compounds. There was a consistent response across males and females and with each test substance.

There were no treatment-related gross findings. Microscopically, minimal centrilobular hypertrophy was observed in both sexes after treatment with all three compounds at 5000 ppm, except in males treated with the *trans* isomer or mixed isomers at 2000 and 5000 ppm. The liver from a small number of rats in the control and 5000 ppm groups of the three compounds was examined using electron microscopy on both semi-thin toluidine blue–stained sections and ultra-thin sections. Some changes, such as a proliferation of smooth endoplasmic reticulum, increased fat and a more frequent occurrence of condensed cells, were found in the liver from sedaxane-treated rats. The changes that were observed were similar with all three test compounds. No treatment-related changes were detected in other tissues except the liver.

In conclusion, the toxicological target organ of *trans* and *cis* isomers and their mixture was the liver. The toxicological profiles of the three compounds were qualitatively similar, and there were very few differences in the incidence or severity of findings observed in response to the three isomer preparations. They all induced drug metabolizing enzyme activity (mainly PROD), although there were some treatment-related differences in testosterone hydroxylation activities. Toxicokinetic analysis indicated higher C_{max} and AUC values for the *trans* isomer than for the *cis* isomer and for the mixture (Peffer & Noakes, 2010).

(e) Toxicity of metabolite CSCD465008

CSCD465008, or 3-(difluoromethyl)-1H-pyrazole-4-carboxylic acid, has been assessed for acute oral toxicity, in vitro genotoxicity (bacterial reverse mutation, in vitro cytogenetics and mammalian gene cell mutation) and repeated-dose oral toxicity for up to 28 days, as summarized in Table 53. CSCD465008 was not acutely toxic by the oral route, was not genotoxic in vitro and did not result in any toxicologically significant effects at dose levels exceeding 1000 mg/kg bw per day, the limit dose for a 28-day toxicity study in rats. These data indicate that CSCD465008 is of lower toxicity than the parent sedaxane.

Table 53. Summary of toxicity studies with CSCD465008

Study	Result	Reference
Acute oral toxicity	LD_{50} > 2000 mg/kg bw	Simon (2008)
Bacterial reverse mutation	Negative	Sokolowski (2008)
In vitro cytogenetics	Negative	Bohnenberger (2008)
Mammalian cell gene mutation (mouse lymphoma)	Negative	Wollny (2008)
28-day dietary toxicity in the rat	NOAEL = 1018 mg/kg bw per day (highest dose tested)	Walraven (2008)

LD_{50}, median lethal dose; NOAEL, no-observed-adverse-effect level

In a 28-day oral toxicity study, CSCD465008 (lot no. MES-103/1; purity 94%), a metabolite of sedaxane, was administered ad libitum via the basal diet for 28 consecutive days to three groups of Crl:WI(Han) rats. Dietary concentrations were 0, 2000, 6000 and 12 000 ppm (equal to 0, 175, 497 and 1018 mg/kg bw per day for males and 0, 176, 525 and 1107 mg/kg bw per day for females, respectively). Each group consisted of five animals of each sex. Following 4 weeks of test diet administration, all animals were euthanized and necropsied. All animals were checked for mortality, clinical examinations and detailed physical examinations. Body weights and feed consumption were recorded. Functional observational battery and locomotor activity data were recorded for all animals. Ophthalmic examinations, clinical pathology evaluations (haematology, coagulation, serum chemistry and urine analysis) were performed on all animals. After necropsies, selected organs were weighed and examined microscopically from all control and the highest dose groups. All gross lesions were examined in all animals. Sections of the liver were collected from all animals for CYP enzyme analysis.

There were no test substance–related effects on clinical observations, body weights, feed consumption, functional observational battery, motor activity, haematology, coagulation, serum chemistry, urine analysis parameters, P450 content, P450 activity (for those enzymes evaluated), ophthalmic examinations and macroscopic or microscopic findings.

The NOAEL was 12 000 ppm (equal to 1018 mg/kg bw per day), the highest dose tested (Walraven, 2008).

In conclusion, CSCD465008 is of low acute toxicity and has no genotoxicity and no toxicity in a 4-week oral toxicity study in rats. The toxicities were weak compared with those of sedaxane.

3. Observation in humans

Analysis of the company's internal database produced no reports of adverse health effects that relate to the synthesis or formulation of sedaxane. As sedaxane has not yet been introduced into the market, there is no information on record of clinical cases and poisoning incidents or observations on exposure of the general population and epidemiological studies. Sedaxane is of low acute toxicity, and no cases of intoxication with sedaxane have yet been observed. No specific monitoring programmes have been performed in humans.

Comments

Biochemical aspects

In rats given [^{14}C]sedaxane labelled in either the phenyl or pyrazole ring as a single oral dose of 1 or 80 mg/kg bw, the radiolabelled material was rapidly and extensively absorbed, based on recoveries in excreta from bile duct–cannulated rats. The times to reach C_{max} were approximately 1 hour and 5–6 hours following the low and high doses, respectively. The mean C_{max} and AUC values for the *trans* isomers were higher than those for the *cis* isomers and their mixture in rats. Approximately 90% of the administered dose was absorbed at both the low and high doses. Radiolabelled material was widely distributed throughout the body within 5 hours. The half-lives of elimination of total radioactivity from different tissues varied from 0.1–0.2 day in brain to 2.0–3.2 days in thyroid. Elimination half-lives from blood ranged from 30 to 40 hours and were generally similar in males and females at both dose levels. Less than 0.8% of the administered dose remained in the body at 96 hours after dosing.

The sedaxane administered to rats was rapidly excreted, predominantly in the faeces (75–88%) and in urine (12–20%). Sedaxane was extensively metabolized in rats by demethylation, hydroxylation, oxidation and conjugation, resulting in many hydroxylated metabolites and metabolites formed by cleavage of the terminal cyclopropyl moiety. The major metabolites have been identified as the *trans-para*-phenol sedaxane and the desmethyl *trans-para*-phenol sedaxane, which, together with the equivalent *cis-para*-phenol isomers of sedaxane, account for approximately half of the administered dose. There appear to be no major sex- or dose-related differences in the qualitative metabolite profile of sedaxane. There is little evidence of any cleavage between the phenyl and pyrazole moieties of the sedaxane molecule. A small amount (< 1%) of a pyrazole amide metabolite of sedaxane also found in plants can be found in bile samples. The phenolic and hydroxy metabolites of sedaxane and desmethyl sedaxane are subject to glucuronic acid, sulfate and glutathione conjugation.

Toxicological data

The oral LD_{50} was 5000 mg/kg bw in rats. Significant clinical signs of toxicity (ruffled fur, hunched posture, sedation, poor coordination, ventral recumbency, deep respiration, rales, salivation and bradypnoea) were observed at lower doses (1750 and 550 mg/kg bw) for a few hours following treatment. The dermal LD_{50} in rats was greater than 5000 mg/kg bw. The 4-hour acute inhalation LC_{50} in rats was greater than 5.2 mg/l. Sedaxane was not irritating to rabbit skin and minimally irritating to rabbit eyes. Sedaxane was not a skin sensitizer in the mouse local lymph node assay.

The short-term oral toxicity of sedaxane was evaluated in mice, rats and dogs, in which the main effects were on body weight gain and liver. In a 28-day study of toxicity in mice, no toxicity was observed at doses up to 7000 ppm (equal to 1268 mg/kg bw per day). In a 90-day dietary toxicity study in mice, the NOAEL was 3500 ppm (equal to 566 mg/kg bw per day), based on a decrease in body weight gain throughout the study in males at 7000 ppm (equal to 1167 mg/kg bw per day).

Two 90-day toxicity studies were conducted in rats, each demonstrating the liver as the target for sedaxane. In the first study, the NOAEL was 1000 ppm (equal to 72.9 mg/kg bw per day), based on lower body weights, centrilobular hepatocyte hypertrophy and pigmentation, and blood chemistry indicating liver dysfunction in males and females at 4000 ppm (equal to 299.6 mg/kg bw per day). In the second study, the NOAEL was 300 ppm (equal to 28.3 mg/kg bw per day), based on reduced body weight and body weight gain and significant decreases in forelimb grip strength at 2000 ppm (equal to 168.0 mg/kg bw per day); liver toxicity was observed at 4000 ppm (equal to 325.1 mg/kg bw per day). The overall NOAEL from these studies was 1000 ppm (equal to 72.9 mg/kg bw per day).

The toxicity of sedaxane administered in capsules was tested in dogs in 90-day and 1-year toxicity studies. The overall NOAEL was 50 mg/kg bw per day, based on reduced body weight gain in females at 150 mg/kg bw per day.

The NOAEL in an 18-month dietary study in mice was 1250 ppm (equal to 157 mg/kg bw per day), based on a decrease in body weight and body weight gain in both sexes at 7000 ppm (equal to 900 mg/kg bw per day). A slightly increased incidence of hepatocellular adenomas and carcinomas combined was observed in male mice at the high dose in comparison with the control group incidence. The NOAEL for equivocal carcinogenicity in mice was 1250 ppm (equal to 157 mg/kg bw per day).

The NOAEL in a 104-week dietary study in rats was 200 ppm (equal to 11 mg/kg bw per day), based on increases in liver weight and histopathological changes (centrilobular hypertrophy) in the liver in males, histopathological changes in the thyroid in males and females, and reduced body weight gain in females at 1200 ppm (equal to 67 mg/kg bw per day). Hepatocellular eosinophilic foci were also increased at 200 ppm in females at 52 weeks, but did not persist at 2 years. Uterine adenocarcinomas were increased at 3600 ppm (equal to 261 mg/kg bw per day). The NOAEL for carcinogenicity was 1200 ppm (equal to 86 mg/kg bw per day), based on uterine tumours in female rats.

Sedaxane was tested for genotoxicity in vitro and in vivo in an adequate range of assays. In none of these assays was there any evidence of genotoxic potential.

The Meeting concluded that sedaxane is unlikely to be genotoxic.

On the basis of the absence of genotoxicity and the fact that equivocal increased incidences of hepatocellular adenomas and carcinomas combined in male mice and uterine endometrial adenocarcinomas in rats occurred only at the highest doses tested, the Meeting concluded that sedaxane is unlikely to pose a carcinogenic risk to humans at dietary exposure levels.

In a multigeneration reproductive toxicity study in rats, the NOAEL for parental toxicity was 500 ppm (equal to 41 mg/kg bw per day), based on significantly reduced body weight gain at 1500 ppm (equal to 120 mg/kg bw per day) in parental generation males. Decreased ovarian follicle counts were observed at 1500 ppm (low and middle doses not examined). Slightly decreased ovary weights were observed at 1500 ppm. The NOAEL for reproductive toxicity was 1500 ppm (equal to 120 mg/kg bw per day). The NOAEL for offspring toxicity was 500 ppm (equal to 43 mg/kg bw per day), based on significantly lower body weights of F_1 generation males during premating at 1500 ppm (equal to 134 mg/kg bw per day).

In a developmental toxicity study in rats, the NOAEL for maternal toxicity was 25 mg/kg bw per day, based on reductions in body weight gain and feed consumption at 100 mg/kg bw per day. The NOAEL for developmental toxicity was 200 mg/kg bw per day, the highest dose tested.

In a developmental toxicity study in rabbits, the NOAEL for maternal toxicity was 100 mg/kg bw per day, based on reductions in body weight gain and feed consumption at 200 mg/kg bw per day.

The NOAEL for developmental toxicity was 100 mg/kg bw per day, based on slight reductions in fetal body weights at 200 mg/kg bw per day.

The Meeting concluded that sedaxane is not teratogenic in rats or rabbits.

The NOAEL in a single-dose neurotoxicity study in rats was 30 mg/kg bw, based on severe loss of general condition, decreased body weight and decreased feed consumption at 250 mg/kg bw.

The NOAEL for systemic toxicity in a 13-week neurotoxicity study was 1000 ppm (equal to 66 mg/kg bw per day), based on decreased body weight, body weight gain, feed consumption and feed efficiency, as well as reduced locomotor activity, at 4000 ppm (equal to 260 mg/kg bw per day).

The Meeting concluded that sedaxane is not neurotoxic.

In an immunotoxicity study in mice, sedaxane was not immunotoxic at doses up to 5500 ppm (equal to 1084 mg/kg bw per day).

A 28-day comparative study of the toxicities of *trans* and *cis* isomers and their mixture in rats demonstrated that their toxicological profiles were qualitatively similar.

The toxicity of a sedaxane plant metabolite (3-(difluoromethyl)-1H-pyrazole-4-carboxylic acid) has been investigated. The LD_{50} value in rats was greater than 2000 mg/kg bw, and the NOAEL in a 28-day oral (gavage) toxicity study in rats was 12 000 ppm (equal to 1018 mg/kg bw per day), the highest dose tested. There was no evidence for genotoxicity in in vitro assays.

No information on medical surveillance or poisoning incidents was available.

The Meeting concluded that the existing database on sedaxane was adequate to characterize the potential hazards to fetuses, infants and children.

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) of 0–0.1 mg/kg bw on the basis of a NOAEL of 200 ppm (equal to 11 mg/kg bw per day) in a 2-year study of toxicity and carcinogenicity in rats, based on reduced body weight gain in females and histopathological changes in the liver in males and in the thyroid in males and females at 1200 ppm (equal to 67 mg/kg bw per day). A safety factor of 100 was applied. The ADI provides a margin of exposure of at least 860 relative to the NOAEL for uterine tumours in rats and at least 1570 for equivocal liver tumour response in mice. Thus, the Meeting considered that sedaxane is not likely to pose a carcinogenic risk to humans at dietary levels of exposure.

An acute reference dose (ARfD) of 0.3 mg/kg bw was established on the basis of a NOAEL of 30 mg/kg bw in a single-dose neurotoxicity study in rats, based on severe loss of general condition, decreased body weight and decreased feed consumption. A safety factor of 100 was applied.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen-month study of toxicity and carcinogenicity[a]	Toxicity	1250 ppm, equal to 157 mg/kg bw per day	7000 ppm, equal to 900 mg/kg bw per day
		Carcinogenicity (equivocal)	1250 ppm, equal to 157 mg/kg bw per day	7000 ppm, equal to 900 mg/kg bw per day

Species	Study	Effect	NOAEL	LOAEL
Rat	Ninety-day studies of toxicity[a,b]	Toxicity	1000 ppm, equal to 72.9 mg/kg bw per day	2000 ppm, equal to 168 mg/kg bw per day
	Twenty-four-month study of toxicity and carcinogenicity[a]	Toxicity	200 ppm, equal to 11 mg/kg bw per day	1200 ppm, equal to 67 mg/kg bw per day
		Carcinogenicity	1200 ppm, equal to 86 mg/kg bw per day	3600 ppm, equal to 261 mg/kg bw per day
	Two-generation study of reproductive toxicity[a]	Reproductive toxicity	1500 ppm, equal to 120 mg/kg bw per day[d]	—
		Parental toxicity	500 ppm, equal to 41 mg/kg bw per day	1500 ppm, equal to 120 mg/kg bw per day
		Offspring toxicity	500 ppm, equal to 43 mg/kg bw per day	1500 ppm, equal to 134 mg/kg bw per day
	Developmental toxicity study[c]	Maternal toxicity	25 mg/kg bw per day	100 mg/kg bw per day
		Developmental toxicity	200 mg/kg bw per day[d]	—
	Single-dose test of neurotoxicity[c]	Toxicity	30 mg/kg bw	250 mg/kg bw
Rabbit	Developmental toxicity study[c]	Maternal toxicity	100 mg/kg bw per day	200 mg/kg bw per day
		Developmental toxicity	100 mg/kg bw per day	200 mg/kg bw per day
Dog	Ninety-day and 12-month studies of toxicity[b,c]	Toxicity	50 mg/kg bw per day	150 mg/kg bw per day

[a] Dietary administration.
[b] Two or more studies combined.
[c] Gavage administration.
[d] Highest dose tested.

Estimate of acceptable daily intake for humans

0–0.1 mg/kg bw

Estimate of acute reference dose

0.3 mg/kg bw

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposure

Critical end-points for setting guidance values for exposure to sedaxane

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid , > 87%
Dermal absorption	No data

Distribution	Widely distributed
Potential for accumulation	None
Rate and extent of excretion	Rapid, > 99.5% within 2 days
Metabolism in animals	Main four metabolites by demethylation, hydroxylation, oxidation and conjugation
Toxicologically significant compounds in animals, plants and the environment	Parent compound and all of the individual isomers
Acute toxicity	
Rat, LD_{50}, oral	5000 mg/kg bw
Rat, LD_{50}, dermal	> 5000 mg/kg bw per day
Rat, LC_{50}, inhalation	> 5.244 mg/l
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Mildly irritating
Dermal sensitization	Not sensitizing (local lymph node assay)
Short-term studies of toxicity	
Target/critical effect	Liver and reduced body weight gain
Lowest relevant oral NOAEL	50 mg/kg bw per day
Lowest relevant dermal NOAEL	1000 mg/kg bw per day
Lowest relevant inhalation NOAEC	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Liver, thyroid and reduced body weight gain
Lowest relevant NOAEL	11 mg/kg bw per day
Carcinogenicity	Equivocal hepatic tumours in mice and uterine tumours in rats; unlikely to pose a carcinogenic risk at dietary exposure levels
Genotoxicity	
	Not genotoxic
Reproductive toxicity	
Target/critical effect	No reproductive toxicity
Lowest relevant reproductive NOAEL	120 mg/kg bw per day (highest dose tested)
Lowest relevant parental NOAEL	41 mg/kg bw per day
Lowest relevant offspring NOAEL	43 mg/kg bw per day
Developmental toxicity	
Target/critical effect	No developmental toxicity
Lowest relevant maternal NOAEL	25 mg/kg bw per day
Lowest relevant developmental NOAEL	100 mg/kg bw per day
Neurotoxicity	
Acute neurotoxicity	Not neurotoxic; 250 mg/kg bw (highest dose tested) NOAEL for toxicity: 30 mg/kg bw
Subchronic neurotoxicity	Not neurotoxic; 260 mg/kg bw per day (highest dose tested)
Other toxicological studies	
Comparative toxicity	Toxicological profile similar for *trans* and *cis* isomers and their mixture in rats
Immunotoxicity	Not immunotoxic; 1084 mg/kg bw per day (highest dose tested)

Medical data	
	No reports of toxicity in workers exposed during manufacture or use

Summary

	Value	Study	Safety factor
ADI	0–0.1 mg/kg bw	Two-year study (rats)	100
ARfD	0.3 mg/kg bw	Single-dose study (rats)	100

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ANNEX 1

Reports and other documents resulting from previous Joint Meetings of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues

1. Principles governing consumer safety in relation to pesticide residues. Report of a meeting of a WHO Expert Committee on Pesticide Residues held jointly with the FAO Panel of Experts on the Use of Pesticides in Agriculture. FAO Plant Production and Protection Division Report, No. PL/1961/11; WHO Technical Report Series, No. 240, 1962.
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This volume contains toxicological monographs that were prepared by the 2012 Joint FAO/WHO Meeting on Pesticide Residues (JMPR), which met in Rome on 11–20 September 2012.

The monographs in this volume summarize the safety data on 11 pesticides that could leave residues in food commodities. These pesticides are ametoctradin, bentazone, chlorfenapyr, dinotefuran, fenpropathrin, fenvalerate, fluxapyroxad, glufosinate-ammonium, MCPA, picoxystrobin and sedaxane. The data summarized in the toxicological monographs served as the basis for the acceptable daily intakes and acute reference doses that were established by the Meeting.

This volume and previous volumes of JMPR toxicological evaluations, many of which were published in the FAO Plant Production and Protection Paper series, contain information that is useful to companies that produce pesticides, government regulatory officers, industrial testing laboratories, toxicological laboratories and universities.

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