Pesticide residues in food — 2011

Joint FAO/WHO Meeting on Pesticide Residues

EVALUATIONS 2011

Part II — Toxicological

Food and Agriculture Organization of the United Nations

World Health Organization

Pesticide residues in food — 2011

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

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Geneva, 20–29 September 2011

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Abbreviations used

20-MCA	20-methylcholanthrene
4-MUGT	4-methylumbelliferone glucuronosyltransferase
4′-OH	2-(4-ethoxyphenyl)-2-methylpropyl 3-(4-hydroxyphenoxy)benzyl ether
4′-OH-PB-acid	3-(4-hydroxyphenoxy) benzoic acid
α-CO	2-(4-ethoxyphenyl)-2-methylpropyl 3-phenoxybenzoate
ABC	adenosine triphosphate–binding cassette
ACh	acetylcholine
AChE	acetylcholinesterase
ACTH	adrenocorticotropic hormone
ADI	acceptable daily intake
AFC	antibody-forming cell
AH	aniline-4-hydroxylase; aniline hydroxylation
AhR	aryl hydrocarbon receptor
a.i.	active ingredient
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AMPA	aminomethylphosphonic acid
AP	aminopyrine demethylase; aminopyrine *N*-demethylation
APTT	activated partial thromboplastin time
AR	androgen receptor
ARfD	acute reference dose
AST	aspartate aminotransferase
ATP	adenosine-5′-triphosphate
AUC	area under the concentration–time curve
BaP	benzo(*a*)pyrene
BQ	benzyloxyquinoline debenzylase
BrdU	5-bromo-2′-deoxyuridine
BROD	benzyloxyresorufin *O*-debenzylase
bw	body weight
CAR	constitutive androstane receptor
CBA	chlorobenzoic acid
cDNA	complementary deoxyribonucleic acid
CDNB	1-chloro-2,4-dinitrobenzene
CHA	chlorohippuric acid
ChE	cholinesterase
CHL	Chinese hamster lung
CHO	Chinese hamster ovary
CI	confidence interval

C_{max}	peak concentration in blood
CoA	coenzyme A
cRNA	complementary ribonucleic acid
Ct	threshold cycle
CT	computed tomography
CYP	cytochrome P450
DCBA	dichlorobenzilic acid
DCBH	dichlorobenzhydrol
DCBP	dichlorobenzophenone
DDD	dichlorodiphenyldichloroethane
DDE	dichlorodiphenyldichloroethene
DDT	dichlorodiphenyltrichloroethane
DE	3-phenoxybenzyl 2-(4-hydroxyphenyl)-2-methylpropyl ether
DHT	dihydrotestosterone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DNCB	dinitrochlorobenzene
DP	3-hydroxybenzyl 2-(4-ethoxyphenyl)-2-methylpropyl ether
dUTP	deoxyuridine triphosphate
EC_{50}	median effective concentration
ECG	electrocardiograph
EMS	ethyl methanesulfonate
eq	equivalent
ER	estrogen receptor
ERα	estrogen receptor alpha
EROD	7-ethoxyresorufin *O*-deethylase
EU	European Union
F	filial generation (e.g. F_0, F_1, F_2)
FAO	Food and Agriculture Organization of the United Nations
FOB	functional observational battery
FSH	follicle-stimulating hormone
GAT	glyphosate-*N*-acetyltransferase
GC-MS	gas chromatography–mass spectrometry
GD	gestation day
GGT	gamma-glutamyltranspeptidase; gamma-glutamyltransferase
GLP	good laboratory practice
GnRH	gonadotropin releasing hormone
GSD	geometric standard deviation
HC	historical control; hepatic cytochrome
HDT	highest dose tested
H&E	haematoxylin and eosin

hERα	human estrogen receptor alpha
HPG	hypothalamic–pituitary–gonadal
HPLC	high-performance liquid chromatography
HPRT	hypoxanthine-guanine phosphoribosyl transferase
IC_{50}	median inhibitory concentration
IgM	immunoglobulin M
ip	intraperitoneal
IPCS	International Programme on Chemical Safety
ISO	International Organization for Standardization
IU	international unit
IUPAC	International Union of Pure and Applied Chemistry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
kat	katal (SI unit of catalytic activity)
LC_{50}	median lethal concentration
LC-MS	liquid chromatography–mass spectrometry
LC-MS/MS	liquid chromatography–tandem mass spectrometry
LD	lactation day
LD_{50}	median lethal dose
LDH	lactate dehydrogenase
LH	luteinizing hormone
LHR	luteinizing hormone receptor
LHRH	luteinizing hormone releasing hormone
LOAEC	lowest-observed-adverse-effect concentration
LOAEL	lowest-observed-adverse-effect level
LOEL	lowest-observed-effect level
LSC	liquid scintillation counting
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
MFO	mixed-function oxidase
MHA	microcytic hypochromic anaemia
MMAD	mass median aerodynamic diameter
MMS	methyl methanesulfonate
MOA	mode of action
m-PB-acid	3-phenoxybenzoic acid
m-PB-alc	3-phenoxybenzyl alcohol
mRNA	messenger ribonucleic acid
MRT	mean residence time
nAChR	nicotinic acetylcholine receptor
NADPH	reduced nicotinamide adenine dinucleotide phosphate

ND	not detected
NG	naphthyl glucuronide
NMR	nuclear magnetic resonance
NOAEC	no-observed-adverse-effect concentration
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
NR	not reported
NS	not significantly different
NTE	neuropathy target esterase
NTP	National Toxicology Program (USA)
OECD	Organisation for Economic Co-operation and Development
OH-DCBP	hydroxyl dichlorobenzophenone
OH-DCBH	hydroxyl dichlorobenzhydrol
o,*p'*-ER-8	1-chloro-1-(2-chlorophenyl)-1-(4-chlorophenyl)-2,2,2-trichloroethane
OPPTS	Office of Prevention, Pesticides and Toxic Substances (USEPA)
OR	odds ratio
P	parental generation (e.g. P_1, P_2)
PAP	*p*-aminophenol
PBS	phosphate-buffered saline
PCE	polychromatic erythrocyte
PCNA	proliferating cell nuclear antigen
PEG	polyethylene glycol
PFC	plaque-forming cell
PNA	*p*-nitroanisole *O*-demethylation
PND	postnatal day
p-NPGT	*p*-nitrophenol glucuronosyltransferase
po	per os
PPARα	peroxisome proliferator-activated receptor alpha
p,*p'*-ER-8	1-chloro-1,1-bis-(4-chlorophenyl)-2,2,2-trichloroethane
ppm	part per million
PPO	protoporphyrinogen IX oxidase
PROD	7-pentoxyresorufin *O*-dealkylase
PT	prothrombin time
PXR	pregnane X receptor
QA	quality assurance
RF	resorufin
RT-PCR	real-time polymerase chain reaction
S9	9000 × *g* rat liver supernatant
SAR	structure–activity relationship
SD	standard deviation

SDS-PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
SGOT	serum glutamic oxaloacetic transaminase (AST)
SGPT	serum glutamic pyruvic transaminase (ALT)
SI	Système international d'unités
SPECT	single-photon emission computed tomography
$t_{½}$	half-life
T_3	triiodothyronine
T_4	thyroxine
TK	thymidine kinase
TLC	thin-layer chromatography
T_{max}	time to reach peak concentration in plasma (C_{max})
TOCP	tri-*o*-tolyl phosphate
TP	thyroid microsomal peroxidase
TRR	total radioactive residues
TSH	thyroid stimulating hormone
TT	thrombotest
TUNEL	terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick end labelling
U	unit
UDPGT	uridine diphosphate glucuronosyltransferase
UDS	unscheduled deoxyribonucleic acid synthesis
USEPA	United States Environmental Protection Agency
V_{avg}	average response amplitude
V_{max}	maximum amplitude of the auditory startle response
v/v	volume per volume
WHO	World Health Organization
WT	wild type
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 Geneva, Switzerland, on 20–29 September 2011.

Eight of the substances evaluated by the WHO Core Assessment Group (acetamiprid, emamectin benzoate, flutriafol, isopyrazam, penthiopyrad, propylene oxide, saflufenacil and sulfoxaflor) were evaluated for the first time. Three compounds (dichlorvos, dicofol and etofenprox) were re-evaluated within the periodic review programme of the Codex Committee on Pesticide Residues (CCPR). The Joint Meeting was also asked to evaluate newly submitted studies on metabolites of glyphosate that are found in genetically modified crops. 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 211*. 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 2011, 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 temporary advisers before the 2011 Joint Meeting. A special acknowledgement is made to those advisers and to the Members of the Joint Meeting who reviewed early drafts of these working papers.

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

ACETAMIPRID

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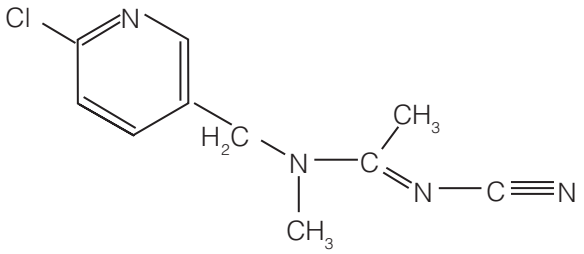
Explanation

Acetamiprid is the International Organization for Standardization (ISO)–approved name for (*E*)-N^1-[(6-chloro-3-pyridyl)methyl]-N^2-cyano-N^1-methyl acetamidine (International Union of Pure and Applied Chemistry). Its Chemical Abstracts Service number is 135410-20-7. Acetamiprid is a neonicotinoid insecticide that is used for the control of sucking-type insects on leafy vegetables, fruiting vegetables, cole crops, citrus fruits, pome fruits, grapes, cotton and ornamental plants and flowers. Acetamiprid is being reviewed for the first time by the Joint FAO/WHO Meeting on Pesticide Residues at the request of the Codex Committee on Pesticide Residues.

All critical studies contained statements of compliance with good laboratory practice (GLP).

The chemical structure of acetamiprid is shown in Figure 1.

Figure 1. Chemical structure of acetamiprid



Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution, elimination and pharmacokinetics

(a) Oral route

To obtain information on the absorption, distribution, rate and route of elimination, metabolism and pharmacokinetics of acetamiprid, a study was performed in adult Sprague-Dawley rats (body weight 154–193 g for males, 134–152 g for females; aged 5–6 weeks at the start of dosing; dosing for 15 days) using [^{14}C]acetamiprid. The radiolabelled test substance (batch No. CFQ8019, chemical purity > 99.9%, radiochemical purity 97.1–97.2%) was sent by the sponsor to the contract research organization. The non-labelled test substance was from lot No. NNI-01, with a chemical purity of greater than 99.9%.

The studies were conducted after oral administration of the test substance for 15 days. In total, five treatment groups (groups I, II, III, IV and V), consisting of 6 rats (3 males and 3 females) in each of the first three groups and 10 rats (5 males and 5 females) in each of the two remaining groups, were used. A single control group (group VI), consisting of four rats (two males and two females), was used.

Groups I, II and III received oral doses of [^{14}C]acetamiprid in 0.9% saline for 15 days at a target dose rate of 1.0 mg/kg body weight (bw). Groups IV and V received oral doses of acetamiprid in 0.9% saline for 14 days followed by a single oral dose of [^{14}C]acetamiprid in 0.9% saline on day 15. The actual dose rate was 0.97–1.01 mg/kg bw for the rats in all five groups. The radiochemical purity of [^{14}C]acetamiprid in the dose solution was determined to be 97.9% by high-performance liquid chromatographic (HPLC) analysis. The dose solution was stable under refrigerated conditions for at

Table 1. Group designation and dose level

Group No.	No. of males/ no. of females	Nominal dose		Frequency	Sacrifice (h)
		mg/kg bw	ml/kg bw		
I	3/3	1.0	1	Daily for 15 days	1
II	3/3	1.0	1	Daily for 15 days	10
III	3/3	1.0	1	Daily for 15 days	96
IV	5/5	1.0	1	Daily for 15 days	96
V	5/5	1.0	1	Daily for 15 days	48
VI	2/2	0	1	Daily for 15 days	96

From Premkumar, Guo & Vegurlekar (1995)

Table 2. [^{14}C]Acetamiprid concentration in blood collected 1 hour post-dosing on days 1, 3, 7 and 15 from rats of group III

Sex of rat		[^{14}C]Acetamiprid concentration in blood (µg/ml)			
		Day 1	Day 3	Day 7	Day 15
Male	Mean	0.590	0.747	0.477	0.606
	± SD	0.130	0.211	0.135	0.073
Female	Mean	0.465	0.491	0.511	0.698
	± SD	0.043	0.060	0.099	0.065

From Premkumar, Guo & Vegurlekar (1995)
SD, standard deviation

least 15 days. The specific activity of the radiolabelled dose solution was determined to be 1.85×10^3 Bq/µg. Group VI was dosed with 0.9% saline only.

Rats of groups I, II and III were sacrificed 1, 10 and 96 hours, respectively, after dosing of [^{14}C]acetamiprid for 15 days. Rats of group IV were sacrificed 96 hours after a single dose of [^{14}C]-acetamiprid for tissue and organ collection. Group V was used only for blood pharmacokinetic analysis (Table 1).

Whole blood was drawn from each rat of group III approximately 1 hour post-dosing on days 1, 3, 7 and 15 to determine the [^{14}C]acetamiprid concentration in blood. The average concentration in blood was in the range of 0.477–0.747 µg/ml in the males and 0.465–0.698 µg/ml in the females. Variation between animals was observed. These results indicate that the blood concentration at 1 hour post-dosing was consistent during the entire dosing period (Table 2).

Whole blood was drawn from each rat of group V at approximately 0.25, 0.5, 1, 2, 3, 4, 5, 7, 9, 12, 24 and 48 hours to determine the [^{14}C]acetamiprid concentration in blood. The mean values for peak concentration (C_{max}), time to C_{max} (T_{max}), absorption half-life ($t_{½\,(ka)}$) and area under the concentration versus time curve at infinity (AUC_{∞}) for the male rats were 0.798 ± 0.111 µg/ml, 2.80 ± 0.637 hours, 1.35 ± 0.825 hours and 8.35 ± 1.12 µg eq·h/ml, respectively. Values for the same parameters in female rats averaged 0.861 ± 0.132 µg/ml, 2.81 ± 0.894 hours, 1.18 ± 0.868 hours and 10.3 ± 2.90 µg eq·h/ml, respectively. The elimination half-lives ($t_{½\,(k)}$) for the male and female rats were 4.42 ± 1.10 hours and 5.56 ± 1.93 hours, respectively. The pharmacokinetic parameters for both sexes did not differ considerably. The T_{max} values in both sexes indicated that the rate of absorption of acetamiprid was rapid, and a maximum blood concentration to possible saturation was achieved in approximately 2–3 hours (Table 3).

Table 3. Mean whole blood pharmacokinetic parameters in rats in group V (dosed for 14 days with non-labelled acetamiprid followed by labelled acetamiprid on day 15)

Sex of rat		C_{max} (µg/ml)	T_{max} (h)	$t_{½\,(ka)}$ (h)	$t_{½\,(k)}$ (h)	AUC_{∞} (µg eq·h/ml)
Male	Mean	0.798	2.80	1.35	4.42	8.35
	± SD	0.111	0.637	0.825	1.10	1.12
Female	Mean	0.861	2.81	1.18	5.56	10.3
	± SD	0.132	0.894	0.868	1.93	2.90

From Premkumar, Guo & Vegurlekar (1995)
eq, equivalent; SD, standard deviation

Table 4. Recovery of administered dose in faeces, urine and cage rinse[a]

Sacrifice time (h)	Group	Sex		% of total administered dose eliminated in			
				Faeces	Urine	Cage rinse	Total
1	I	Male	Mean	31.0	53.4	7.57	92.0
			± SD	0.56	5.24	2.97	2.89
		Female	Mean	21.9	58.0	10.7	90.6
			± SD	2.43	5.39	1.42	5.08
10	II	Male	Mean	29.8	56.6	7.32	93.7
			± SD	3.12	6.90	1.95	3.56
		Female	Mean	25.2	59.3	6.98	91.5
			±SD	6.00	4.11	2.21	0.92
96	III	Male	Mean	32.0	61.4	3.92	97.4
			± SD	4.08	0.64	0.73	4.87
		Female	Mean	27.5	56.0	7.93	91.4
			± SD	1.42	2.45	2.67	1.51
96	IV	Male	Mean	35.3	64.8	5.86	106
			± SD	5.97	6.99	2.81	5.44
		Female	Mean	28.7	62.1	11.3	102
			± SD	4.30	5.32	3.72	5.31

From Premkumar, Guo & Vegurlekar (1995)
SD, standard deviation
[a] Animals of groups I, II and III were treated with [^{14}C]acetamiprid for 15 days and sacrificed 1, 10 and 96 hours after the administration of the 15th dose. Animals of group IV were treated with acetamiprid for 14 days, and on the 15th day, a single dose of [^{14}C]acetamiprid was given, 96 hours after which the animals were sacrificed.

The elimination results indicate that most acetamiprid (53–65%) was excreted in the urine. The excretion in urine and cage rinse combined amounted to 61–73%. The results also indicate that acetamiprid was absorbed rapidly (within 1 hour) from the gastrointestinal tract, as greater than 90% of the administered dose was eliminated from the gastrointestinal tract within 1 hour after dosing. No difference was observed in elimination of test substance between chronic administration of acetamiprid for 14 days followed by a single administration of radiolabelled acetamiprid on day 15 (group IV) and chronic administration of radiolabelled acetamiprid for 15 days (groups I, II and III). The amount of administered radioactivity eliminated in faeces was lower for females (22–29%) than for males (30–35%) (Table 4).

The whole blood, liver, kidney, lung, pancreas, spleen, heart, brain, testes (male), ovary (female), skeletal muscles, inguinal fat (white), skin with hair, thyroid, bone, adrenal glands, gastrointestinal tract with contents, cage rinses and residual carcasses were collected from each rat of groups I, II, III and IV. All collected samples were not composited but kept and analysed separately to account for the material balance for each rat.

Radioactivity, after administration of the last chronic dose, was detected at the earliest sampling point (1 hour) in all the tissues collected from each rat. The radioactivity in most tissues was the highest at 1 hour post-dosing and declined rapidly thereafter (groups II and III). The T_{max} for [^{14}C]acetamiprid in the male and female rats indicated that the rate of absorption was rapid, and a maximum blood concentration (~0.8 µg/ml) to possible saturation was achieved in approximately 2–3 hours. The levels of [^{14}C]acetamiprid residue in tissues collected at 1 hour post-dosing confirm the results obtained from the pharmacokinetic analysis.

[^{14}C]Acetamiprid residue levels seen in tissues collected 10 hours post-dosing (group II) were found to be substantially lower than residue levels in tissues collected 1 hour post-dosing. The elimination half-life ($t_{½(k)}$) for both sexes indicated that the rate of elimination was rapid. The levels of [^{14}C]acetamiprid residues in tissues collected at 10 hours post-dosing confirm the results obtained from the pharmacokinetic studies.

[^{14}C]Acetamiprid residue levels seen in tissues collected 96 hours post-dosing (group III) were found to be very low compared with the levels observed in the tissues collected at 1 hour and 10 hours post-dosing. The elimination half-life ($t_{½(k)}$) for both sexes was between 4 and 6 hours post-dosing, indicating that the rate of elimination was rapid and that retention of residue in tissues after chronic administration was minimal.

The highest radioactivity levels were observed in the gastrointestinal tract, liver and kidney in both sexes at all sacrifice times. The lowest concentration was observed in bone and white fat. The residue levels observed were higher in all tissues of rats chronically treated with [^{14}C]acetamiprid for 15 days (group III) compared with the rats in group IV, which received a single final dose of [^{14}C]-acetamiprid following 14 days of non-labelled acetamiprid doses. The residue levels observed in the tissues of rats sacrificed 96 hours after the last dose were very low (0.01–0.1 part per million [ppm]), as most of the administered dose (> 90%) was eliminated through the urine and faeces (Table 5).

The total administered radioactivity recovered in groups I, II, III and IV was in the range of 91.7–106%, whereas recovery in group V (the pharmacokinetics group) was 71.7% and 85.6% in males and females, respectively (Table 6). The loss of urine samples during a series of bleeding procedures is a possible explanation for the low recovery in group V.

The study described in this report was conducted in compliance with GLP. A quality assurance (QA) statement was attached (Premkumar, Guo & Vegurlekar, 1995).

To ascertain the effect of administration of acetamiprid in single low and high doses, the absorption, distribution, metabolism and excretion of acetamiprid in rats were investigated. [Pyridine-2,6-^{14}C]acetamiprid was intravenously or orally administered to five male and five female rats in groups A, B and D at dose levels of 1.0, 1.0 and 50 mg/kg bw, respectively. In group CN-B, the metabolism study of [cyano-^{14}C]acetamiprid was performed at a dose level of 1.0 mg/kg bw. Group A was for the determination of the absorption rate by calculation from the excretion rate and metabolite analysis. Groups B, D and CN-B were for blood levels, tissue distribution, metabolite analysis and excretion rate. The chemical structure and position of the label on the test substance are as shown in Figure 2. The study design is as described in Table 7.

In groups B and D, the absorption in the rats was rapid. The maximum concentrations in the blood were observed at 0.5–2 hours after administration at 0.91 mg/kg bw for males and 1.01 mg/kg bw for females (low dose: ring label, group B) and at 3–7 hours after administration at 40.50 mg/kg bw for males and 31.46 mg/kg bw for females (high dose: ring label, group D).

Table 5. Distribution of [^{14}C]acetamiprid residues in various tissues collected from rats at 1, 10 and 96 hours post-dosing (dosed with [^{14}C]acetamiprid for 15 days)

Tissues	Sex		Concentration of residues (ppm)		
			1 h post-dosing, group I	10 h post-dosing, group II	96 h post-dosing, group III
Gastrointestinal tract	Male	Mean	4.48	2.40	0.010
		± SD	0.82	0.14	0.002
	Female	Mean	3.79	1.91	0.011
		± SD	0.59	0.40	0.001
Liver	Male	Mean	1.62	0.78	0.014
		± SD	0.05	0.28	0.003
	Female	Mean	1.86	0.69	0.011
		± SD	0.09	0.39	0.000
Kidney	Male	Mean	1.43	0.79	0.028
		± SD	0.05	0.26	0.005
	Female	Mean	1.48	0.66	0.023
		± SD	0.09	0.32	0.001
Heart	Male	Mean	0.81	0.39	0.006
		± SD	0.05	0.13	0.001
	Female	Mean	1.00	0.38	0.006
		± SD	0.07	0.26	0.001
Lung	Male	Mean	0.76	0.39	0.009
		± SD	0.06	0.13	0.001
	Female	Mean	0.98	0.38	0.008
		± SD	0.08	0.26	0.003
Blood	Male	Mean	0.75	0.36	0.015
		± SD	0.04	0.10	0.008
	Female	Mean	0.92	0.33	0.007
		± SD	0.04	0.20	0.000
Thyroid	Male	Mean	0.80	0.39	0.000
		± SD	0.04	0.13	0.000
	Female	Mean	0.94	0.37	0.000
		± SD	0.08	0.25	0.000
Spleen	Male	Mean	0.66	0.31	0.004
		± SD	0.04	0.11	0.001
	Female	Mean	0.81	0.31	0.004
		± SD	0.05	0.21	0.001
Adrenals	Male	Mean	0.64	0.35	0.010
		± SD	0.09	0.19	0.002
	Female	Mean	1.23	0.42	0.015
		± SD	0.07	0.26	0.007
Muscle	Male	Mean	0.63	0.31	0.006
		± SD	0.05	0.10	0.001
	Female	Mean	0.81	0.35	0.007
		± SD	0.06	0.22	0.001

Table 5 (continued)

Tissues	Sex		Concentration of residues (ppm)		
			1 h post-dosing, group I	10 h post-dosing, group II	96 h post-dosing, group III
Testes	Male	Mean	0.60	0.30	0.003
		± SD	0.04	0.10	0.001
Ovaries	Female	Mean	0.46	0.23	0.008
		± SD	0.13	0.14	0.004
Skin	Male	Mean	0.58	0.31	0.106
		± SD	0.07	0.10	0.033
	Female	Mean	0.73	0.33	0.067
		± SD	0.05	0.20	0.002
Pancreas	Male	Mean	0.70	0.26	0.002
		± SD	0.17	0.07	0.001
	Female	Mean	0.83	0.25	0.002
		± SD	0.05	0.17	0.001
Brain	Male	Mean	0.59	0.25	0.002
		± SD	0.04	0.07	0.001
	Female	Mean	0.75	0.24	0.002
		± SD	0.02	0.18	0.001
Bone	Male	Mean	0.45	0.17	0.003
		± SD	0.05	0.05	0.001
	Female	Mean	0.58	0.20	0.004
		± SD	0.07	0.14	0.001
Fat	Male	Mean	0.27	0.11	0.007
		± SD	0.02	0.02	0.002
	Female	Mean	0.47	0.17	0.010
		± SD	0.08	0.11	0.002

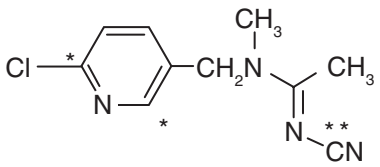
From Premkumar, Guo & Vegurlekar (1995)
SD, standard deviation

Table 6. Mass balance: total average percentage of administered radioactivity dose recovered in samples collected in various groups

Group of animals	Timing of sample collection (h)	Average % of administered dose	
		Males	Females
I	1	101	99.7
II	10	98.5	95.5
III	96	97.6	91.7
IV	96	106.1	103
V	48	71.7	85.6

From Premkumar, Guo & Vegurlekar (1995)

Figure 2. Chemical structure and label position of test substance



(* labelled position for ring -^{14}C-NI-25)
(** labelled position for CN-^{14}C-NI-25)

Table 7. Study design

Group	Mode of administration	Target dose level (mg/kg bw)	Experiment targeted	Test substance	Number of animals	
					Male	Female
A	Single intravenous	1.0	1. Excretion rate 2. Quantitative analysis of metabolites	Ring-^{14}C-acetamiprid	5	8
B	Single oral (low dose)	1.0	1. Blood levels 2. Excretion rate 3. Quantitative analysis of metabolites	Ring-^{14}C-acetamiprid	5	5 (for each of experiments 1, 2 and 3)
					9	9 (for experiment 4)
			4. Tissue distribution 5. Biliary excretion		3	3 (for experiment 5)
D	Single oral (high dose)	50	1. Blood levels 2. Excretion rate 3. Quantitative analysis of metabolites	Ring-^{14}C-acetamiprid	5	5 (for each of experiments 1, 2 and 3)
			4. Tissue distribution		9	9 (for experiment 4)
CN-B	Single oral (low dose)	1.0	1. Blood levels 2. Excretion rate 3. Quantitative analysis of metabolites	CN-^{14}C-acetamiprid	5	5

From Tanoue & Mori (1997a)

The absorption rate of acetamiprid following oral administration was calculated using the following equation, based on urinary excretion rates in oral and intravenous administrations at the low dose:

$$\text{Absorption rate} = \frac{\text{Urinary excretion rate following oral administration}}{\text{Urinary excretion rate following intravenous administration}} \times 100$$

The calculations are shown in Table 8. Thus, the absorption rates were more than 95%. This shows that acetamiprid is easily absorbed in rats.

Table 8. Calculation of absorption rates

Period	Male	Female
Days 0–1	(76.28 ÷ 78.92) × 100 = 96.7%	(73.16 ÷ 76.00) × 100 = 96.3%
Days 0–4	(81.07 ÷ 81.59) × 100 = 99.4%	(79.33 ÷ 79.73) × 100 = 99.5%

From Tanoue & Mori (1997a)

Table 9. Blood concentration of parent substance equivalents and half-life in rats after oral administration of acetamiprid

Group	Blood concentration at the time (h) after administration (mg/kg)											
	0.25	0.5	1	2	3	4	5	7	9	12	24	48
Males												
B	0.55	0.78	0.88	0.81	0.74	0.66	0.58	0.40	0.27	0.14	0.02	< 0.02
C	16.2	23.5	31.4	38.4	39.3	39.9	38.1	33.6	29.0	23.0	5.2	0.3
CN-B	0.64	0.89	0.97	0.95	0.89	0.86	0.79	0.62	0.47	0.30	0.06	0.01
Females												
B	0.79	1.00	1.00	0.88	0.80	0.72	0.63	0.46	0.32	0.20	0.04	< 0.02
C	8.1	15.5	22.4	25.6	28.7	30.3	29.0	27.2	23.6	21.6	9.0	0.4
CN-B	0.53	0.86	0.97	0.96	0.91	0.86	0.78	0.59	0.43	0.26	0.05	0.01

From Tanoue & Mori (1997a)

Table 10. Average C_{max}, range in T_{max} and half-life values of acetamiprid in rats

Group	Sex	$t_{½}$ (h)	C_{max} [a] (mg/kg)	T_{max} [b] (h)
B	Male	7.11	0.91	0.5–2.0
	Female	5.84	1.01	0.5–1.0
D	Male	8.07	40.50	3.0–5.0
	Female	15.03	31.46	3.0–7.0
CN-B	Male	5.90	0.97	1.0
	Female	11.29	0.97	1.0–2.0

From Tanoue & Mori (1997a)
[a] Average C_{max} of five individual values.
[b] Range in T_{max} of five individual values.

After acetamiprid reached its maximum concentrations in the blood, its levels decreased linearly and rapidly. The half-lives of the radioactivity were 5.84–7.11 hours for group B and 8.07–15.03 hours for group D. Similar to group B, the absorption in group CN-B was rapid, and the maximum concentrations in the blood were 0.97 mg/kg for both sexes at 1–2 hours after administration. The blood levels then decreased linearly and rapidly, with half-lives of the radioactivity of 5.90–11.29 hours (Tables 9 and 10).

In group A, the rates of excretion in the urine and faeces 1 day after dosing were 76.00–78.92% and 11.44–11.90% of the initially administered radioactivity, respectively. Rates of excretion in the urine and faeces 1 day after dosing in group B were 73.16–76.28% and 9.91–11.10%, respectively, whereas those in group CN-B were 75.15–79.24% and 4.12–4.56%, respectively. In group D, 72.84% and 56.39% of the total radioactivity were excreted in the urine of males and females, respectively, 1 day after dosing, and 6.13% and 10.20% of the total radioactivity were excreted into the faeces of males and females, respectively. In all of the groups, total excretion rates (i.e. the sum of the excretion

Table 11. Excretion rate of radioactivity in rats after administration of acetamiprid

Group	% of initially administered radioactivity									
	Urine				Faeces				Residual in body	Sum
	Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4		
Males										
A	78.92	1.89	0.56	0.22	11.44	3.32	0.67	0.12	0.63	97.78
B	76.28	4.09	0.46	0.23	9.91	1.53	0.15	0.05	0.42	93.13
D	72.84	11.98	1.17	0.44	6.13	6.39	0.36	0.08	0.74	100.13
CN-B	79.24	9.58	1.05	0.40	4.56	0.63	0.05	0.05	0.96	96.55
Females										
A	76.00	2.40	0.99	0.35	11.90	4.04	0.88	0.22	0.48	97.26
B	73.16	4.63	0.90	0.64	11.10	2.21	0.22	0.26	0.52	93.64
D	56.39	15.20	1.33	0.92	10.20	6.61	0.43	0.09	0.58	91.74
CN-B	75.15	10.93	1.37	0.90	4.12	0.88	0.13	0.06	0.84	94.38

From Tanoue & Mori (1997a)

in urine and faeces) were more than 90% in a 4-day period, and the residual radioactivity in the body was less than 1% of the dose. Because faecal excretion of radioactivity was also observed in group A with intravenous dosing, biliary excretion was suggested. The absorption rates of acetamiprid were all more than 95%, as calculated from the urinary excretion rates in groups A and B (Table 11).

Tissue concentrations in groups B and D were investigated 1, 5, 10 and 96 hours and 5, 14, 24 and 96 hours after administration, respectively, and the half-lives of radioactivity in the tissues were calculated. In a short time after dosing, the radioactivity was widely distributed in the body, but the concentrations in bone and fat were clearly low compared with the blood concentration. In contrast, the adrenal (group B: 1.344–2.409 mg/kg at 1 hour; group D: 51.88–62.87 mg/kg at 5 hours), thyroid (group B: 1.345–1.493 mg/kg at 1 hour; group D: 64.72–68.13 mg/kg at 5 hours), liver (group B: 1.651–1.711 mg/kg at 1 hour; group D: 61.34–62.02 mg/kg at 5 hours) and kidney (group B: 1.458–1.777 mg/kg at 1 hour; group D: 52.55–55.66 mg/kg at 5 hours) had higher concentrations than the blood (group B: 0.771–0.803 mg/kg at 1 hour; group D: 31.46–34.77 mg/kg at 5 hours). The rate of disappearance of radioactivity in the tissues was nearly the same as that in the blood. The blood concentrations in groups B and D were 0.001 mg/kg and 0.07 mg/kg, respectively, at 96 hours after dosing. The half-lives of the radioactivity in the tissues ranged from 2.9 to 7.9 hours (group B) and from 6.0 to 8.5 hours (group D). There were no tissues that were presumed to have accumulated the substance. Similarly to groups B and D, the tissue concentrations in groups A and CN-B were low 96 hours after dosing (Tables 12–15).

In summary, acetamiprid orally dosed in rats was rapidly absorbed and widely distributed into the tissues via blood. The majority of the radioactivity was excreted in the urine through the kidney and in the faeces via bile. The disappearance of the radioactivity from the body of the rat was rapid, and there were no tissues that are presumed to accumulate the compound. No differences in the sexes were observed. This study meets the requirements for GLP, and a QA statement was attached (Tanoue & Mori, 1997a).

A biliary excretion study was conducted using Sprague-Dawley bile duct–cannulated rats approximately 10–12 weeks old at dosing. Four male and four female bile duct–cannulated rats received single doses of [^{14}C]acetamiprid in 0.9% saline through an intragastric cannula. The average dose rates were 1.02 and 1.07 mg/kg bw for the male and female rats, respectively. The radiochemical

Table 12. Tissue concentration of parent substance equivalents in rats after oral administration of acetamiprid (ring label, group B)

	Mean tissue concentration (mg/kg)			
	1 h	5 h	10 h	96 h
Males				
Spleen	0.750	0.438	0.217	0.001
Heart	0.828	0.507	0.255	0.001
Bone	0.470	0.285	0.146	0.001
Lung	0.864	0.507	0.254	0.001
Adrenal	1.344	0.784	0.394	0.001
Sciatic nerve	0.786	0.502	0.252	0.001
Pancreas	0.858	0.503	0.251	0.001
Thyroid	1.493	0.556	0.251	0.002
Whole blood	0.771	0.458	0.221	0.001
Brain	0.677	0.383	0.179	0.001
Liver	1.711	0.918	0.409	0.002
Kidney	1.777	1.268	0.521	0.003
Muscle	0.755	0.467	0.219	0.001
Fat	0.359	0.216	0.093	0.003
Testis	0.754	0.430	0.215	0.000
Skin	0.711	0.430	0.226	0.002
Carcass	0.874	0.516	0.304	0.009
Females				
Spleen	0.801	0.500	0.185	0.001
Heart	0.901	0.616	0.217	0.001
Bone	0.461	0.302	0.209	0.001
Lung	0.858	0.547	0.213	0.002
Adrenal	2.409	1.361	0.296	0.002
Ovary	0.822	0.519	0.183	0.001
Sciatic nerve	0.685	0.570	0.226	0.002
Pancreas	0.914	0.578	0.209	0.001
Thyroid	1.345	0.840	0.467	0.004
Whole blood	0.803	0.505	0.190	0.001
Brain	0.712	0.437	0.150	0.001
Liver	1.651	0.960	0.355	0.002
Kidney	1.458	0.965	0.392	0.003
Muscle	0.800	0.541	0.184	0.001
Fat	0.365	0.219	0.083	0.001
Skin	0.762	0.494	0.184	0.002
Carcass	0.818	0.612	0.262	0.016

From Tanoue & Mori (1997a)

Table 13. Distribution of parent substance equivalent radioactivity in tissues in rats after oral administration of acetamiprid (ring label, group B)

	% of initially administered radioactivity			
	1 h	5 h	10 h	96 h
Males				
Spleen	0.18	0.12	0.05	0.00
Heart	0.34	0.20	0.09	0.00
Lung	0.39	0.24	0.12	0.00
Adrenal	0.03	0.02	0.01	0.00
Pancreas	0.28	0.15	0.08	0.00
Thyroid	0.01	0.01	0.00	0.00
Brain	0.63	0.34	0.17	0.00
Liver	5.59	3.43	1.70	0.01
Kidney	1.66	1.31	0.51	0.00
Testis	0.74	0.43	0.22	0.00
Carcass[a]	73.04	46.14	28.87	0.40
Females				
Spleen	0.21	0.11	0.05	0.00
Heart	0.37	0.22	0.08	0.00
Lung	0.44	0.32	0.11	0.00
Adrenal	0.09	0.06	0.01	0.00
Ovary	0.05	0.04	0.01	0.00
Pancreas	0.32	0.17	0.05	0.00
Thyroid	0.01	0.01	0.00	0.00
Brain	0.86	0.53	0.18	0.00
Liver	6.16	3.92	1.57	0.01
Kidney	1.42	0.94	0.40	0.00
Carcass[a]	73.60	56.24	23.99	0.50

From Tanoue & Mori (1997a)

[a] "Carcass" includes residual tissues after necropsy (bone, nervous tissue, blood, muscle, fat and skin).

purity of [^{14}C]acetamiprid in the dose solution was determined to be 97.1% by HPLC analysis. One male and one female rat were dosed with placebo (0.9% saline, containing no test substance).

A steady increase in [^{14}C]acetamiprid residue level was observed in bile from 3 to 12 hours post-dosing, with the highest amount (percentage of administered dose) at 12 hours post-dosing in both male and female rats. The average recovery of the administered dose in bile over a 48-hour period was 19.9% ± 1.47% in the male rats and 18.6% ± 0.62% in the female rats. Recovery of the [^{14}C]acetamiprid residues excreted in bile accounted for less than 20% of the total administered dose, suggesting that bile is not a predominant excretory pathway in either the male or the female rats. The absorption of the test substance and the extent of first-pass metabolism/presystemic elimination were not significantly different between the sexes.

The average recovery of the administered dose in faeces over a 48-hour period was 6.72% ± 3.36% in the male rats and 5.84% ± 0.86% in the female rats. The average recovery of the administered dose in urine over a 48-hour period was 24.3% ± 5.22% in the male rats and 36.9% ± 3.80% in the female rats. In the male and female rats, the sum of urine plus cage rinses, 60.2% ± 5.20% and 64.4% ± 2.86%, respectively, accounted for the major residues, suggesting that most of the administered dose was excreted in urine.

The average recovery of the administered dose in liver at 48 hours post-dosing was 0.22% ± 0.13% in the male rats and 0.18% ± 0.18% in the female rats. The average recovery of the administered

Table 14. Tissue concentration of parent substance equivalents in rats after oral administration of acetamiprid (ring label, group D)

	Tissue concentration (mg/kg)			
	5 h	14 h	24 h	96 h
Males				
Spleen	35.67	15.35	4.83	0.04
Heart	37.70	17.57	5.77	0.06
Bone	22.12	10.24	3.51	0.04
Lung	43.65	17.38	5.45	0.06
Adrenal	62.87	24.22	7.87	0.15
Sciatic nerve	47.18	14.15	6.55	0.08
Pancreas	36.83	15.96	5.27	0.04
Thyroid	68.13	49.85	7.89	0.13
Whole blood	31.46	15.45	5.05	0.07
Brain	27.80	12.28	3.60	0.03
Liver	61.34	25.84	9.70	0.16
Kidney	52.55	27.02	11.25	0.21
Muscle	33.37	14.77	4.90	0.06
Fat	15.97	7.41	2.05	0.10
Testis	30.54	13.88	4.62	0.06
Skin	30.26	14.90	5.95	0.18
Carcass	39.01	22.49	7.43	0.74
Females				
Spleen	34.49	11.85	4.93	0.04
Heart	37.39	13.76	5.54	0.05
Bone	20.91	8.13	3.09	0.07
Lung	40.39	13.40	5.34	0.05
Adrenal	51.88	17.44	8.82	0.05
Ovary	34.56	12.24	5.14	0.03
Sciatic nerve	50.84	15.78	6.51	0.01
Pancreas	39.40	13.77	5.69	0.03
Thyroid	64.72	18.42	7.83	0.08
Whole blood	34.77	10.71	5.30	0.07
Brain	28.89	9.64	3.92	0.06
Liver	62.02	21.16	9.20	0.12
Kidney	55.66	23.37	11.32	0.18
Muscle	34.36	13.97	5.15	0.07
Fat	17.73	6.00	3.25	0.15
Skin	31.61	11.70	5.33	0.16
Carcass	39.07	21.99	7.43	0.96

From Tanoue & Mori (1997a)

Table 15. Distribution of parent substance equivalent radioactivity in tissues of rats after oral administration of acetamiprid (ring label, group D)

	% of initially administered radioactivity			
	5 h	14 h	24 h	96 h
Males				
Spleen	0.17	0.08	0.02	0.00
Heart	0.33	0.15	0.05	0.00
Lung	0.78	0.16	0.05	0.00
Adrenal	0.03	0.01	0.01	0.00
Pancreas	0.22	0.09	0.04	0.00
Thyroid	0.01	0.01	0.00	0.00
Brain	0.53	0.23	0.07	0.00
Liver	4.30	2.34	1.16	0.02
Kidney	0.96	0.54	0.23	0.00
Testis	0.57	0.20	0.09	0.00
Carcass[a]	66.47	44.06	14.83	0.71
Females				
Spleen	0.19	0.06	0.02	0.00
Heart	0.35	0.11	0.04	0.00
Lung	0.45	0.15	0.06	0.00
Adrenal	0.04	0.01	0.01	0.00
Ovary	0.05	0.01	0.01	0.00
Pancreas	0.28	0.10	0.04	0.00
Thyroid	0.01	0.00	0.00	0.00
Brain	0.70	0.21	0.09	0.00
Liver	4.60	2.25	1.05	0.02
Kidney	1.15	0.50	0.23	0.00
Carcass[a]	69.72	43.08	14.70	0.56

From Tanoue & Mori (1997a)

[a] "Carcass" includes residual tissues after necropsy (bone, nervous tissue, blood, muscle, fat and skin).

dose in the gastrointestinal tract at 48 hours post-dosing was 0.46% ± 0.34% in the male rats and 0.33% ± 0.23% in the female rats. These results indicate that an insignificant amount of acetamiprid (< 1% in the collected tissues) was absorbed into the liver or remained in the gastrointestinal tract in both the male and female rats.

The total recoveries of the administered dose in the three male rats were 93.2%, 92.8% and 89.6%, respectively. The total recoveries of the administered dose in the three female rats were 94.9%, 93.5% and 91.2%, respectively.

The study described in this report was conducted in compliance with GLP. A QA statement was attached (Premkumar & Guo, 1995).

(b) Dermal route

The extent of absorption of acetamiprid was studied following application of 70% wettable powder containing [^{14}C]acetamiprid (purity 97.5%) to the skin of male Crl: CD(SD)BR rats. The animals were approximately 8 weeks old upon arrival and weighed 176–216 g (preliminary phase) and

Table 16. Dose administration

Phase	Group	Mean dose levels	
		mg/animal	μg/cm^2
Preliminary	1	0.0128	1.03
Preliminary	2	1.26	101
Definitive	4	0.0136	1.09
Definitive	5	0.119	9.53
Definitive	6	1.13	90.2

From Cheng (1997)

143–203 g (definitive phase). Target dose levels were 1, 10 and 100 μg/cm^2. Actual dose levels were 0.0136 mg/animal (1.09 μg/cm^2), 0.119 mg/animal (9.53 μg/cm^2) and 1.13 mg/animal (90.2 μg/cm^2).

A preliminary phase, consisting of two groups of four animals each, was conducted to evaluate and establish test material application and skin washing techniques. In the preliminary phase, male rats were dermally dosed at two levels (0.0128 mg/animal and 1.26 mg/animal) (Table 16).

In the definitive phase, three groups of 24 rats per group were dermally dosed with [^{14}C]-acetamiprid at three dose levels (Table 16). A control group of two rats received only the vehicle (1% carboxymethylcellulose aqueous solution). Urine and faeces were collected from each rat. Immediately before sacrifice, the skin at the application site was washed. Four rats per time point from each dose group were sacrificed at 0.5, 1, 2, 4, 10 and 24 hours; the control rats were sacrificed at 24 hours. At sacrifice, blood was collected by cardiac puncture.

Among the treated groups, the mean total recovery of radioactivity ranged from 96.6% to 102%, with most of the radioactivity (63.9–87.5%) in the skin wash. Radioactivity in the skin at the application site accounted for 10.2–32.2% of the applied radioactivity. Radioactivity in blood, excreta and carcasses accounted for less than 6.50% of the applied radioactivity.

The amounts of radioactivity found in the blood, eliminated in the excreta and retained in the carcass were considered to result from direct dermal absorption of [^{14}C]acetamiprid. Within groups, amounts of dermal absorption increased with increasing exposure time. The highest absorption was detected at the longest exposure time, 24 hours post-dosing, and accounted for 4.27% (0.581 μg), 6.34% (7.54 μg) and 2.82% (31.9 μg) for the 1.09, 9.53 and 90.2 μg/cm^2 dose groups, respectively. The sum of direct absorption and amount of radioactivity remaining in the skin at the application site was considered to be indirect absorption. The amounts of indirect absorption were 3–5 μg, 25–37 μg and 118–197 μg for the 1.09, 9.53 and 90.2 μg/cm^2 dose groups, respectively. The highest concentration of radioactivity in blood was 0.001 ppm for the 1.09 μg/cm^2 dose group at 24 hours post-dosing, 0.019 ppm and 0.010 ppm for the 9.53 μg/cm^2 dose group at 10 and 24 hours post-dosing, respectively, and 0.041 ppm for the 90.2 μg/cm^2 dose group at 24 hours post-dosing. The amount of direct absorption of acetamiprid in rats was proportional at the two lower dose levels and appeared to reach saturation at the highest dose level.

The study complied with GLP, and a QA statement was attached (Cheng, 1997).

1.2 Biotransformation

In order to undertake the qualitative and quantitative analysis of metabolites, the group IV animals of the Premkumar, Guo & Vegurlekar (1995) study described above were used. This group IV corresponds to group C of United States Environmental Protection Agency (USEPA) guidelines.

In this group, five males and five females were orally administered a daily dose of non-labelled acetamiprid for 14 days followed by a single dose of radiolabelled acetamiprid on day 15. The urine and faeces were collected once on day 14 and then at 24-hour intervals after administration of the [^{14}C]acetamiprid dose solution until sacrifice.

Qualitative analysis of metabolites was performed by thin-layer co-chromatography with unlabelled reference substances. The unknown metabolite was identified by liquid chromatography–tandem mass spectrometry (LC-MS/MS) as the glycine conjugate of IC-O (abbreviated as IC-O-Gly).

The major radioactive compounds in the excreta of rats were acetamiprid itself (males: 5.21%; females: 7.41%), demethylated compound IM-2-1 (males: 15.48%; females: 20.39%), nicotinic acid derivative IC-O (males: 11.12%; females: 8.01%) and IC-O glycine conjugate IC-O-Gly (males: 10.10%; females: 10.32%). In addition, MeS-IC-O, IM-1-4, IM-2-4, IM-O, IM-1-3 and IM-2-3 were detected, but they accounted for less than 2% of the dose. There were several unknown compounds in urine, and the maximum abundance of an unknown compound in the "others" fraction was 1.0%.

It was considered that the major metabolic routes of acetamiprid in rats are the production of IM-2-1 by *N*-demethylation, the production of IC-O by detachment of the cyanoacetamide side-chain from IM-2-1, and the production of IS-1-1 and IS-2-1 by detachment of the cyanoacetamide side-chain from acetamide and IM-2-1, respectively.

The study described in this report was conducted in compliance with GLP. A QA statement was attached (Premkumar, Guo & Vegurlekar, 1995).

A similar picture of metabolites was also observed in the study of Tanoue & Mori (1997a) described above. In that study, radioactive compounds in the excreta of rats were identified and analysed quantitatively. The major compounds identified were acetamiprid itself (males: 6.10%; females: 5.63%), demethylated compound IM-2-1 (males: 19.51%; females: 19.00%) and nicotinic acid derivative IC-O (males: 28.19%; females: 25.52%) in group B; acetamiprid (males: 7.75%; females: 7.34%), IM-2-1 (males: 24.48%; females: 21.37%) and IC-O (males: 27.11%; females: 27.63%) in group D; and acetamiprid (males: 4.16%; females: 6.12%), IM-2-l (males: 13.39%; females: 18.98%) and IC-O (males: 28.13%; females: 24.73%) in group A. Acetamiprid (males: 3.98%; females: 4.51%), IM-2-1 (males: 16.95%; females: 16.56%), IS-1-1 (males: 13.15%; females: 16.45%) and IS-2-1 (males: 35.61%; females: 30.23%) were detected as the main compounds in group CN-B. IS-1-1 and IS-2-1 were thought to be generated by cleavage of the side-chains of acetamiprid and IM-2-1. In addition, IC-O-Gly, MeS-IC-O, IM-1-4, IM-2-4, IM-O, IM-1-3 and IM-2-3 were detected in groups A, B and D, but each at less than 4% of the dose.

The main metabolic pathways of acetamiprid in rats were the transformation to IM-2-1 by demethylation and further to IC-O after cleaving IS-1-1 and IS-2-1 from acetamiprid and IM-2-1, respectively (Tanoue & Mori, 1997a).

The study described in this report was conducted in compliance with GLP. A QA statement was attached (Tanoue & Mori, 1997b).

Another metabolism study of acetamiprid in rats was performed to determine whether IM-1-5, a metabolite, was found in excreta. In each group, three male rats 7 weeks of age were dosed orally with [pyridine-2,6-^{14}C]acetamiprid by a single gavage at a low dose of 1 mg/kg bw or a high dose of 51 mg/kg bw. The excretion balance was also investigated until 96 hours after dosing.

Excretion was rapid, and most of the radioactivity (85.7% of the initially administered radioactivity for the low dose within 24 hours and 90.4% of the initially administered radioactivity for the high dose within 48 hours) was eliminated, especially in the urine.

The quantification of urinary and faecal metabolites was carried out by HPLC for each specimen collected during the 24 hours after dosing. The amount of IM-1-5 was estimated, and it account-

ed for 4.5% and 0.4% of the initially administered radioactivity in the low-dose and high-dose urine, respectively. In the faeces of both doses, no IM-1-5 was detected. The major metabolite in the excreta was IC-O, which accounted for 35.9% and 33.6% of the initially administered radioactivity at the low dose and high dose, respectively. IM-2-1 was the second major metabolite, with 18.5% and 9.3% of the initially administered radioactivity at the low dose and high dose, respectively. Acetamiprid was detected in amounts of 5.2% of the initially administered radioactivity at the low dose and 4.5% of the initially administered radioactivity at the high dose. Similar metabolite profiles were observed for the two doses.

The study was conducted as per GLP, and a QA statement was attached (Saito, 2003).

The proposed metabolic pathway is shown in Figure 3.

2. Toxicological studies

2.1 Acute toxicity

The oral median lethal dose (LD_{50}) of acetamiprid was 198 and 184 mg/kg bw in male and female mice, respectively. In different rat strains, the LD_{50} was in the range of 140–417 mg/kg bw. These studies demonstrated dose-related reversible toxic signs, such as crouching, tremor and convulsion, mydriasis and sensitivity (e.g. lateral position, salivation and ataxia), appearing within 10 minutes to 3 hours after administration and disappearing after 1 day. The dermal LD_{50} in rats was greater than 2000 mg/kg bw, with a dose of 2000 mg/kg bw causing neither mortality nor systemic toxicity. No local skin reaction was observed at the application site. When acetamiprid was administered to rats by inhalation through nose-only exposure, the median lethal concentration (LC_{50}) was greater than 1.15 mg/l of air (4-hour exposure), with a mass median aerodynamic diameter (MMAD) of 8 µm, the highest concentration tested, without any noted clinical signs. However, when rats were exposed whole body to acetamiprid with an MMAD of 5 µm, the LC_{50} was greater than 0.30 mg/l, the highest dose tested. Mydriasis in many rats and tremor and convulsion in a few rats were observed, which disappeared after 1 day. Acetamiprid was not an irritant in a study of ocular and dermal irritation in rabbits or a dermal sensitizer in the Magnusson and Kligman maximization test in guinea-pigs.

The results of acute toxicity studies with acetamiprid are summarized in Table 17. All the studies were conducted as per Organisation for Economic Co-operation and Development (OECD), USEPA and Japanese Ministry of Agriculture, Forestry and Fisheries guidelines and complied with GLP.

2.2 Short-term studies of toxicity

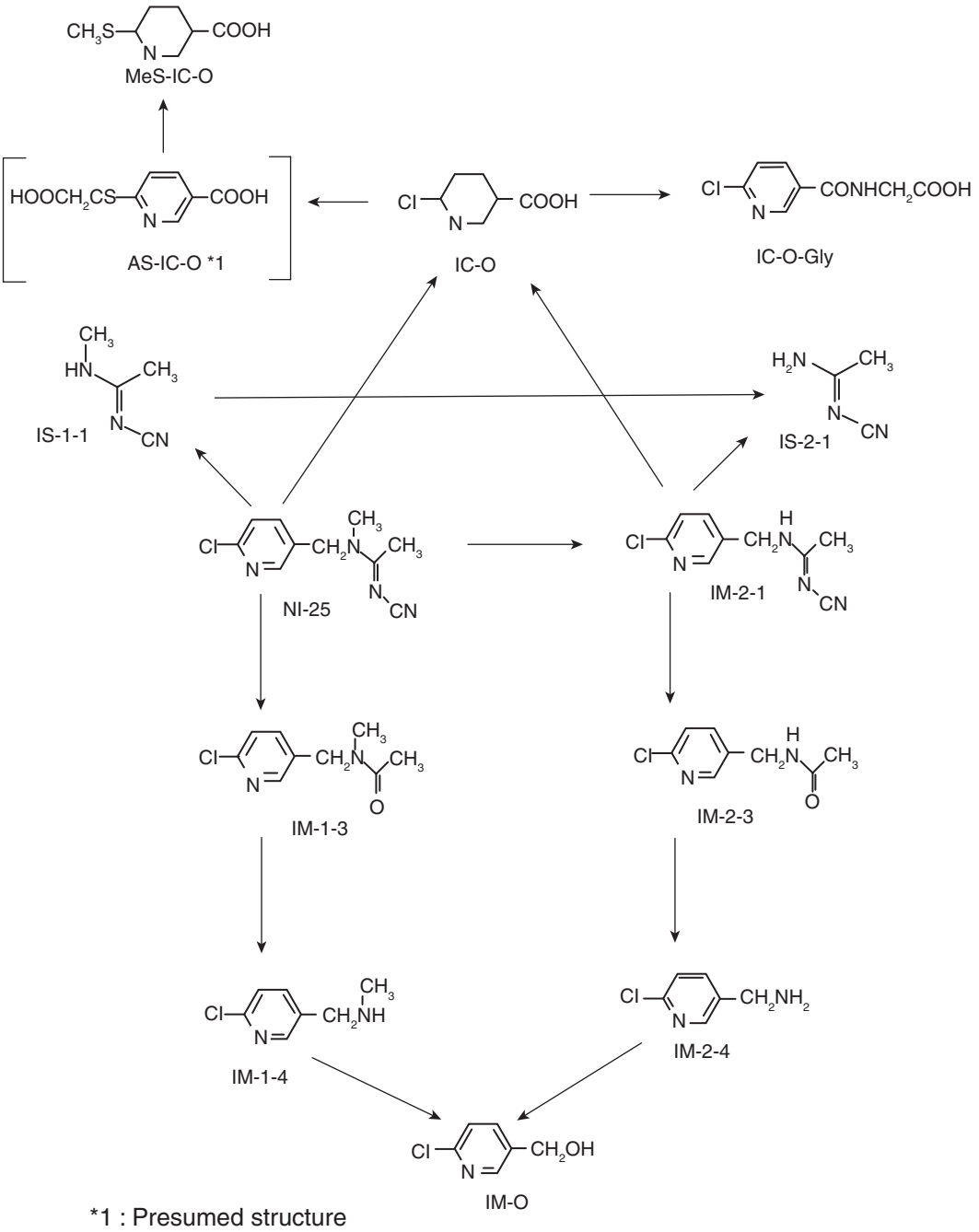
Short-term studies of oral toxicity in mice, rats and dogs were conducted.

(a) Oral administration

Mice

The subchronic toxicity of acetamiprid (lot No. 5910017-(Tox-470), purity 99.2%) in Cij: CD-1(ICR) mice (7 weeks of age) was assessed. The test compound was offered in the diet to 100 mice (10 of each sex per group) at a dose level of 0, 400, 800, 1600 or 3200 ppm for a period of 13 weeks. On the day of study initiation, the weights of animals were 34.4 ± 1.5 g (mean ± standard deviation [SD]) (range 31.2–37.8 g) for males and 25.8 ± 1.2 g (23.5–28.1 g) for females. Mean test compound consumptions for the 400, 800, 1600 and 3200 ppm groups were 53.2, 106.1, 211.1 and 430.4 mg/kg bw per day in males and 64.6, 129.4, 249.1 and 466.3 mg/kg bw per day in females, respectively.

Figure 3. Proposed metabolic pathway of acetamiprid in rats



The five females of the 3200 ppm group showed tremor at weeks 4–13, and two of them died, one at week 8 and the other at week 10. Two females, one each from the control and 800 ppm groups, died as a result of sampling accidents during the haematological examination performed at week 13. Two males of the 3200 ppm group died at week 12; one of them was euthanized in extremis because of decreased body weight compared with the initial body weight of this animal. These male animals did not show any tremor during in-life observations.

Decreased body weights were noted in both sexes of the 1600 and 3200 ppm groups at the study termination, and mean body weights of these groups were 87% and 66% of control values in males and 82% and 64% of control values in females, respectively (Table 18). Decreases in feed

Table 17. Summary of acute toxicity studies with acetamiprid

Species	Strain	Sex	Route	Batch No.; purity (%)	LD_{50} (mg/kg bw)	LC_{50} (mg/l)	Results	Reference
Mouse	Crj:ICR,SPF	M + F	Oral	NNI-02; 99.46	M: 198 F: 184	—	[a]	Mochizuki & Goto (1992)
Rat	Crj:CD(SD), SPF	M + F	Oral	NNI-02; 99.46	M: 217 F: 146	—	[b]	Mochizuki & Kanaguchi (1992)
Rat	Crj:CD(SD), SPF	M + F	Oral	NFG-02; 99.9	M: 417 F: 314	—	[c]	Takaori (1997b)
Rat	Crj:CD(SD), IGS, SPF	M + F	Oral	NKP-194-07; 99.9 (suspended in corn oil)	M: 195 F: 140–200	—	[d]	Fujii (2002a)
Rat	Crj:CD(SD), SPF	M + F	Dermal	NNI-02; 99.46	> 2000	—	[e]	Mochizuki & Fujii (1998)
Rat	Crj:CD(SD), SPF	M + F	Dermal	NFG-02; 99.9	> 2000	—	[e]	Takaori (1997a)
Rat	Crj:CD(SD)	M + F	Inhalation 4 h (whole-body exposure)	NNI-03; 99.57	—	> 0.30 (dust; MMAD 5 µm)	[f]	Saika (1994)
Rat	Sprague-Dawley	M + F	Inhalation 4 h (nose-only exposure)	NFG-02; 99.9	—	> 1.15 (dust; MMAD 8 µm)	[g]	Jackson (1997)
Rabbit	New Zealand White	M	Primary dermal irritation	NNI-02; 99.46	—	—	Non-irritant	Mochizuki & Goto (1993a)
Rabbit	New Zealand White	M	Eye irritation	NI-25; 99.46	—	—	Non-irritant	Mochizuki & Goto (1993b)

Table 17 (continued)

Species	Strain	Sex	Route	Batch No.; purity (%)	LD_{50} (mg/kg bw)	LC_{50} (mg/l)	Results	Reference
Guinea-pig	Dunkin/ Hartley	F	Skin sensitization effects (guinea-pig maximization)	NNI-02; 99.46	—	—	Non-sensitizer	Mochizuki (1994a)
Guinea-pig	Hartley	M + F	Skin sensitization effects (delayed contact hypersensitivity)	NFG-02; 99.9	—	—	Non-sensitizer	Coleman (1997)

F, female; LC_{50}, median lethal concentration; LD_{50}, median lethal dose; M, male; MMAD, mass median aerodynamic diameter

[a] At a dose of 100 mg/kg bw, crouching was observed for 20 minutes to 3 hours in males and for 20 minutes to 1 hour in females after administration. At 150–400 mg/kg bw in both sexes, most mice showed tremors for 10 minutes to 3 hours after administration. Additionally, in 150–400 mg/kg bw males and 290–400 mg/kg bw females, a few mice showed convulsion for 20 minutes to 1 hour after administration. All toxic signs disappeared within 1 day after the administration. In some surviving females of the two highest doses, the body weight decreased on day 1 and recovered afterwards. Six out of 27 dead mice revealed dark-reddish lung on necropsy.

[b] No toxic signs were observed in 100 mg/kg bw males and 80 mg/kg bw females. In 150–304 mg/kg bw males and in 100–230 mg/kg bw females, most rats showed crouching for 3 hours to 1 day after administration. In 150–510 mg/kg bw males and 100–510 mg/kg bw females, most rats showed tremors for 3 hours to 1 day after administration. A few rats showed low sensitivity, lateral position, prone position, salivation, urinary incontinence and ataxia for 60 minutes to 1 day. All toxic signs disappeared within 2 days after administration. Three rats out of 37 dead revealed dark-reddish lung on necropsy.

[c] Clinical signs noted in the treated rats were lacrimation (1 rat in 100 mg/kg bw group), mydriasis, tremor, clonic convulsion, prone position and lateral position. These signs appeared shortly after administration, and their incidences reached a maximum at 60 or 180 minutes. No abnormality was observed at gross necropsy.

[d] Mydriasis and tremor were observed in all dose groups. Clonic convulsions were observed in males at 200, 280 and 560 mg/kg bw and in females at 280, 400 and 560 mg/kg bw. These signs appeared shortly after administration and reached a maximum at 60 or 180 minutes. All deaths occurred within 1 day after administration. There were no treatment-related macroscopic observations.

[e] No toxic signs were observed, and no deaths occurred.

[f] Mydriasis in many rats and tremor and convulsion in a few rats were observed. These toxic signs disappeared after 1 day. Alopecia and crust were observed in a few rats after 1–4 days.

[g] Highest concentration tested. No clinical signs were noted during exposure.

Table 18. Mean body weight and comparison with control values

Dietary concentration (ppm)	Mean body weight (g) and comparison with control values (%)					
	Males			Females		
	Week 0	Week 6	Week 13	Week 0	Week 6	Week 13
0	34.26 (100)	39.30 (100)	41.22 (100)	25.78 (100)	30.44 (100)	33.64 (100)
400	34.45 (100)	38.55 (98)	41.71 (101)	25.77 (100)	29.47 (97)	30.73 (91)
800	34.20 (100)	38.30 (97)	40.13 (97)	25.89 (100)	29.92 (98)	31.37 (93)
1600	34.48 (99)	35.14 (89)a	35.83 (87)b	25.84 (100)	26.93 (88)b	27.45 (82)a
3200	34.46 (100)	26.95 (69)b	27.34 (66)c	25.73 (100)	21.43 (70)b	21.61 (64)c

From Nukui & Ikeyama (1992a)
Significantly different from the control group: "a" $P < 0.05$; "b" $P < 0.01$; "c" $P < 0.001$ (multiple comparison procedure)

consumption values (grams per animal per day) were noted in both sexes at 3200 ppm and in females at 1600 ppm. No effect of test compound treatment on feed consumption value per unit body weight (grams per kilogram body weight per day) was evident, except for a statistically significant decrease at week 1 and an increase at week 9 in the 3200 ppm males (Table 19). Feed efficiency values in both sexes of the 3200 ppm group were decreased from the control group and attained statistical significance occasionally throughout the study, except that the values increased over the control group at week 13.

All animals received ophthalmological examinations prior to study initiation and at week 12 of the study. No test compound treatment–related effects were evident in treated groups at the week 12 examination.

The haematological examination was not performed on the 3200 ppm group because of marked growth depression in both sexes. No effects of test compound treatment were evident in treated groups, except for a statistically significant decrease in haemoglobin concentration seen in the 1600 ppm group females.

Statistically significant decreases in total cholesterol concentration were seen in the females of the 800, 1600 and 3200 ppm groups at study termination. There was also a decrease in total cholesterol concentration in males at 3200 ppm. Glucose concentration was decreased in both sexes at 3200 ppm and in males only at 1600 ppm. Statistically significant increases were noted in blood urea nitrogen level in males and females at the high dose (3200 ppm); however, no effects on creatinine levels were seen at this dose (Table 20).

No statistically significant changes were noted in creatinine, total bilirubin, total protein, albumin, albumin to globulin ratio, sodium, potassium, chloride, calcium, phosphorus, alkaline phosphatase, lactate dehydrogenase or creatine kinase. Considering that proteinuria and renal lesions were not seen in these groups, the cause of the increased blood urea nitrogen may be prerenal.

A statistically significant decrease in urinary pH was found in the 3200 ppm group males at the week 12 examination. The mechanism of this decrease was not apparent.

Statistically significant increases were noted in the liver to body weight ratios of males and females at 800 ppm and above. In the high-dose (1600 and 3200 ppm) groups, decreases in organ weights were found for many organs, which were considered to be attributed to the decreased body weights of the groups (Table 21).

Mean relative liver weight ratios were increased. The liver lesion having a test compound relationship in both sexes was centrilobular hepatocellular hypertrophy. This hypertrophy could be due to induction of microsomal enzymes.

Table 19. Mean feed consumption and mean acetamiprid consumption

Dietary concentration (ppm)	Mean feed consumption (weeks 1–13)				Mean acetamiprid consumption (weeks 1–13)	
	g/animal per day		g/kg bw per day		mg/animal per day	
	Males	Females	Males	Females	Males	Females
0	5.0	4.8	129.7	159.1	0.0	0.0
400	5.2	4.7	132.9	161.6	53.2	646
800	5.1	4.8	132.6	161.8	106.1	129.4
1600	4.6	4.1	132.0	155.7	211.1	249.1
3200	3.6	3.1	134.5	145.7	430.4	466.3

From Nukui & Ikeyama (1992a)

Table 20. Statistically significant changes in blood chemistry examination

	Parameters	Dietary concentration (ppm)			Sex
Decrease	Glucose		1600	3200	Male
				3200	Female
	Total cholesterol			3200	Male
		800	1600	3200	Female
Increase	Urea nitrogen			3200	Male + female
	Alanine aminotransferase			3200	Male + female
	Aspartate aminotransferase			3200	Male
	Cholinesterase			3200	Male

From Nukui & Ikeyama (1992a)

Table 21. Statistically significant changes in organ weights

	Organ	Measurement[a]	Dietary concentration (ppm)			Sex
Decrease	Brain	Absolute		1600	3200	Female
	Thymus	Absolute			3200	Male + female
	Lung	Absolute			3200	Male + female
	Spleen	Absolute, relative			3200	Male + female
		Absolute		1600		Male
	Kidney	Absolute			3200	Male
		Absolute		1600	3200	Female
	Adrenal	Absolute			3200	Female
	Ovary	Absolute, relative			3200	Female
Increase	Brain	Relative			3200	Male + female
	Lungs	Relative			3200	Male + female
	Liver	Relative	800	1600	3200	Male + female
	Adrenal	Relative			3200	Male
	Testis	Relative		1600	3200	Male

From Nukui & Ikeyama (1992a)

[a] Absolute organ weight or organ weight relative to body weight.

Necropsy revealed no compound-related lesions. Histologically, dose-related centrilobular hepatocellular hypertrophy was seen in males and females of the 3200 ppm groups. In animals that died during the study, pulmonary congestion and thymic atrophy were observed, along with some lesions seen in terminally sacrificed animals.

Based on the results mentioned above, the effects of acetamiprid offered in the diet to Crj:CD-1(ICR) mice were tremor, decreased body weight gain, decreased feed consumption, decreased haemoglobin concentration, decreased serum total cholesterol and glucose levels, decreased urinary pH, increased liver to body weight ratios and centrilobular hepatocellular hypertrophy. The no-observed-adverse-effect level (NOAEL) was considered to be 400 ppm (equal to 53.2 mg/kg bw per day), based on a significant decrease in total cholesterol level in females at 800 ppm (equal to 106.1 mg/kg bw per day).

The study complied with GLP, and a QA statement was attached (Nukui & Ikeyama, 1992a).

Rats

In a 13-week dietary study, the subchronic toxicity of acetamiprid (lot No. 31-0023-HY(Tox-447), purity > 99%) in Crj:CD(SD) rats (6 weeks of age) was assessed. The test compound was offered in the diet to 120 rats (10 of each sex per group) at a dose level of 0, 50, 100, 200, 800 or 1600 ppm for 13 weeks. On the day of study initiation, 60 males weighing 178.5 ± 8.5 g (mean ± SD) (range 157.5–190.5 g) and 60 females weighing 147.6 ± 7.0 g (range 137.3–161.1 g) were assigned to one of the six groups by a computerized randomization procedure. Mean test compound consumptions of the 50, 100, 200, 800 and 1600 ppm groups were 3.1, 6.0, 12.4, 50.8 and 99.9 mg/kg bw per day in males and 3.7, 7.2, 14.6, 56.0 and 117.1 mg/kg bw per day in females, respectively.

There were no signs of reaction to treatment in any treated animals. All animals survived throughout the study. Mean weights for high-dose (800 and 1600 ppm) males and females were significantly less than those of the control group throughout the study. Mean body weights of these groups were 91% and 87% of control values in males and 89% and 79% of control values in females, respectively (Table 22). Feed consumption values of these groups were lower than control values. Feed efficiency values of the 1600 ppm animals were occasionally decreased.

Ophthalmological examination did not reveal any effects of test compound treatment in the 1600 ppm group animals at week 12–13 examinations. No effects of test compound treatment were found in the haematological parameters in the treated group animals at the 13-week examination. Increases in total cholesterol concentration were seen in the 1600 ppm group males and females at study termination, reaching statistical significance in the males. On urinalysis, although a statistically significant decrease in urinary ketone body was found in the 1600 ppm males, it was considered to be an occasional occurrence.

Statistically significant increases were noted in the liver to body weight ratios of the 800 and 1600 ppm males and females. Mean absolute thyroid weights and thyroid to body weight ratio were increased to a statistically significant degree only in the males of the two highest dose groups (800 and 1600 ppm), but not in females. Because of the decreases in final body weight in the high-dose groups, statistically significant increases in organ weight ratios (testis, brain, lung, heart and kidney) and decreases in absolute organ weights (adrenal, heart and kidney) were noted in the high-dose groups; however, no microscopic findings were correlated with the weight changes (Table 23).

Necropsy revealed no compound-related lesions. Histologically, dose-related centrilobular hepatocellular hypertrophy was seen in the males and females of the 800 ppm groups and above. Although the thyroid weights were increased in males of the high-dose group, there were no correlating microscopic observations to explain the weight increase. Other microscopic changes were occasionally seen in the control and treated groups, but they were unrelated to the treatment of test compound. One mammary adenocarcinoma, which is considered to be spontaneous, was seen in a female of the 200 ppm group. Other lesions were occasionally seen in the liver (microgranuloma), stomach

Table 22. Mean body weight and comparison with control values

Dietary concentration (ppm)	Mean body weight (g) and comparison with control values (%)					
	Males			Females		
	Week 0	Week 6	Week 13	Week 0	Week 6	Week 13
0	178.5 (100)	424.6 (100)	504.9 (100)	147.7 (100)	267.0 (100)	307 (100)
50	178.4 (100)	417.6 (98)	498.5 (98)	147.9 (100)	261.7 (98)	295.0 (96)
100	178.4 (100)	414.6 (98)	507.6 (100)	147.5 (100)	263.0 (99)	304.9 (99)
200	178.6 (100)	427.8 (101)	525.3 (104)	147.7 (100)	265.5 (99)	315.0 (102)
800	178.5 (100)	384.2 (90)a	463.7 (91)	147.5 (100)	240.7 (90)a	273.6 (89)
1600	178.8 (100)	364.4 (86)b	441.4 (87)a	147.4 (100)	209.6 (79)b	242.5 (79)b

From Nukui & Ikeyama (1992b)
Significantly different from the control group: a, $P < 0.05$; b, $P < 0.01$ (multiple comparison procedure)

Table 23. Statistically significant changes in organ weights

Change	Organ	Measurement[a]	Dietary concentration (ppm)		Sex
Increase	Thyroid	Absolute, relative	800	1600	Male
	Liver	Relative	800	1600	Male + female
	Testis	Relative	800	1600	Male
	Brain, lungs, kidney (left)	Relative		1600	Male + female
	Kidney (right)	Relative	800	1600	Female
	Heart	Relative		1600	Female
Decrease	Adrenal	Absolute		1600	Female
	Heart	Absolute		1600	Female
	Kidney (left)	Absolute		1600	Female

From Nukui & Ikeyama (1992b)
[a] Absolute organ weight or organ to body weight ratio.

(haemorrhage), kidney (lymphocytic inflammatory infiltrate, calcium deposition and regenerative tubules), prostate (lymphocytic inflammatory infiltrate), thymus (haemorrhage), conjunctiva (cell infiltrate), skin (follicular atrophy), spleen (microgranuloma) and lung (cell infiltrate).

Based on the results mentioned above, the effects of acetamiprid offered in the diet to rats were decreased body weight gain, decreased feed consumption, increased serum total cholesterol level, increased liver to body weight ratio and centrilobular hepatocellular hypertrophy in the 800 and 1600 ppm groups (equal to 50.8 and 99.9 mg/kg bw per day, respectively). The NOAEL was 200 ppm (equal to 12.4 mg/kg bw per day).

The study complied with GLP, and a QA statement was attached (Nukui & Ikeyama, 1992b).

Dogs

To assess potential toxicity, acetamiprid (lot No. NNI-02, purity 99.46%) was administered via dietary admixture to 16 Beagles (two of each sex per group) at a dose level of 250, 500, 1000 or 3000 ppm for a period of 28 days. Control animals (two of each sex per group) received standard laboratory diet. Animals receiving 3000 ppm started with a dose of 125 ppm daily up to test day 12, after which the dose was increased to 3000 ppm, as it was decided that an increase in the low dose level was necessary because of a lack of significant toxicity at the 1000 ppm dose level. After the concentration was increased to 3000 ppm, animals in this group continued to receive the 3000 ppm

Table 24. Test substance intake

Group	Dietary concentration (ppm)	Test substance intake (mg/kg bw per day)	
		Males	Females
II	125	3–5	4–6
III	250	8–9	6–10
IV	500	16–18	15–22
V	1000	22–34	28–43
II	3000	21–81	23–82

From Auletta (1992)

dose level for a 28-day period. At the initiation of the study, the animals were approximately 6 months old, and the mean weights were 11 kg (range 9.8–12.6 kg) for males and 9 kg (range 7.3–8.6 kg) for females.

Physical observations, body weight and feed consumption measurements, haematology and biochemistry studies were performed on all animals pretest and at the termination of the treatment period. After at least 28 days of treatment, all survivors were sacrificed, selected organs were weighed and organ to body weight and organ to brain weight ratios were calculated. Complete gross postmortem examinations were conducted on all animals.

Analysis of preliminary batches of diet confirmed that the mixing procedure used produced homogeneous mixtures of acetamiprid in the diet and that this material was stable in the diet when stored at room temperature for at least 14 days after preparation. Analyses confirmed that diets administered were of the appropriate concentrations. Individual test substance intake values, based on feed consumption values and nominal dietary concentrations, ranged as shown in Table 24. The wide range of values for the group receiving 3000 ppm reflects the wide variability of feed consumption values for this group.

All animals survived throughout the study. Acetamiprid administration produced marked weight losses (11–19% of pretest weights), correlating with decreased feed consumption and an emaciated appearance, in dogs receiving 3000 ppm and possible slight decreases in body weight gain and/or feed consumption in animals receiving 1000 ppm. Body weight and feed consumption patterns for animals receiving dietary concentrations of 125, 250 or 500 ppm were comparable to patterns seen in control animals.

No effect of acetamiprid administration on haematology or biochemistry parameters was evident at any dose level. Organ weights were comparable for control and treated groups or exhibited differences consistent with body weight losses; macroscopic postmortem examinations were unremarkable.

Based on the body weight losses seen in animals receiving a dietary concentration of 3000 ppm and the absence of a definitive effect at 1000 ppm, the NOAEL for dietary administration of acetamiprid to dogs for 4 weeks under the conditions of this study is 1000 ppm (equal to a range of 22–43 mg/kg bw per day).

The study complied with GLP, and a QA statement was attached (Auletta, 1992).

In a second dog study, acetamiprid (lot No. NNI-01, purity 99.36%) was administered orally via dietary admixture to 24 Beagle dogs (four of each sex per group) at a dose level of 320, 800 or 2000 ppm (equal to 13, 32 and 58 mg/kg bw per day for males and 14, 32 and 64 mg/kg bw per day for females, respectively) for a period of at least 3 months (Table 25). Control animals (four of each sex per group) received untreated standard laboratory diet. At the initiation of the study, the animals

Table 25. Test substance intake

Group	Dietary concentration (ppm)	Mean test substance intake (mg/kg bw per day)			
		Males		Females	
		Range	Average	Range	Average
II	320	12–14	13	13–14	14
III	800	30–34	32	28–35	32
IV	2000	35–81	58	42–85	64

From Auletta (1994a)

were approximately 6 months old, and the mean weights were 8.6 kg (range 7.6–10 kg) for males and 8.0 kg (range 7.3–8.6 kg) for females.

Physical observations, ophthalmoscopic examinations, body weight and feed consumption measurements, haematology, clinical chemistry and urinalyses were performed on all animals pretest and at selected intervals during the treatment period. After at least 3 months of treatment, all survivors were sacrificed, selected organs were weighed and organ to body weight and organ to brain weight ratios were calculated. Complete gross postmortem examinations and histopathological evaluation of selected tissues were conducted on all animals.

Analysis of preliminary mixes confirmed that the preparation procedure used for this study produced homogeneous mixtures and that the test substance was stable in the diet for at least 14 days. Analyses conducted during the treatment period confirmed that diets of appropriate concentrations were administered.

All animals survived throughout the study. Dose-related effects on body weights were evident early in the study in the groups receiving the 800 and 2000 ppm concentrations. All high-dose (2000 ppm) dogs lost weight during the 1st week of study, and all but one lost weight between the 1st and 2nd weeks. Little or no weight change occurred in these groups during the subsequent weeks. Mean weights and weight gains for mid-dose (800 ppm) dogs were slightly lower than control values during the first 4 weeks and generally close to control values thereafter. Body weights and body weight gains for low-dose (320 ppm) males and females were comparable to concurrent control values throughout the study (Figures 4 and 5).

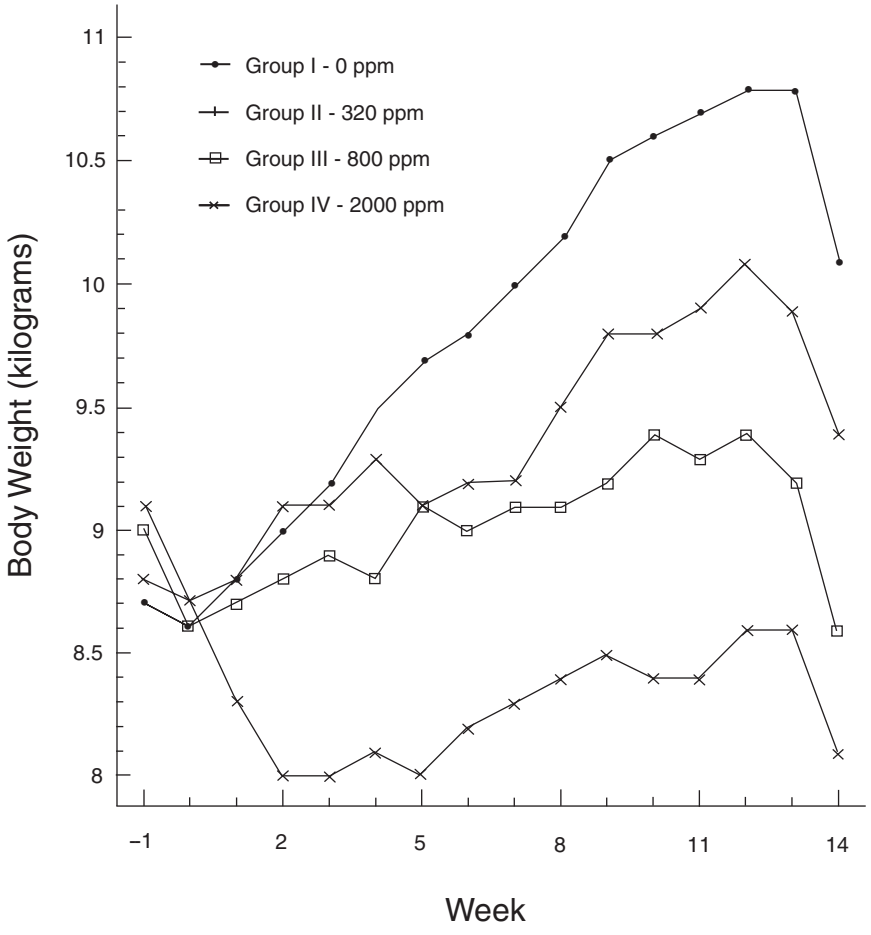
Feed consumption alterations were consistent with body weight patterns. Feed consumption values for high-dose males were lower than control values during the first 6 weeks of treatment; values for high-dose females were lower than control values through week 10. Values for mid-dose females were slightly lower than control values for weeks 1 through 3, but generally comparable to control values thereafter. Values for mid-dose males and low-dose males and females were comparable to control values.

No effect of acetamiprid administration was evident from clinical observations or ophthalmological examinations. No clear effects were seen in clinical laboratory studies (haematology, biochemistry and urinalysis).

Alterations suggestive of an effect of acetamiprid administration on organ weights consisted of a dose-related, statistically significant decrease in mean thyroid/parathyroid weights, relative to control, for all three treated groups of females. Mean thyroid/parathyroid to body weight ratios for all groups and the mean thyroid/parathyroid to brain weight ratio for the high-dose group were also statistically significantly lower than the control values. Similar differences were not apparent in males treated at any dose level.

Although decreased thyroid/parathyroid weights were seen for females at all dose levels (320, 800 and 2000 ppm), the toxicological significance of this finding, in the absence of morphological

Figure 4. Group mean body weight values in male dogs



Source: Auletta (1994a)

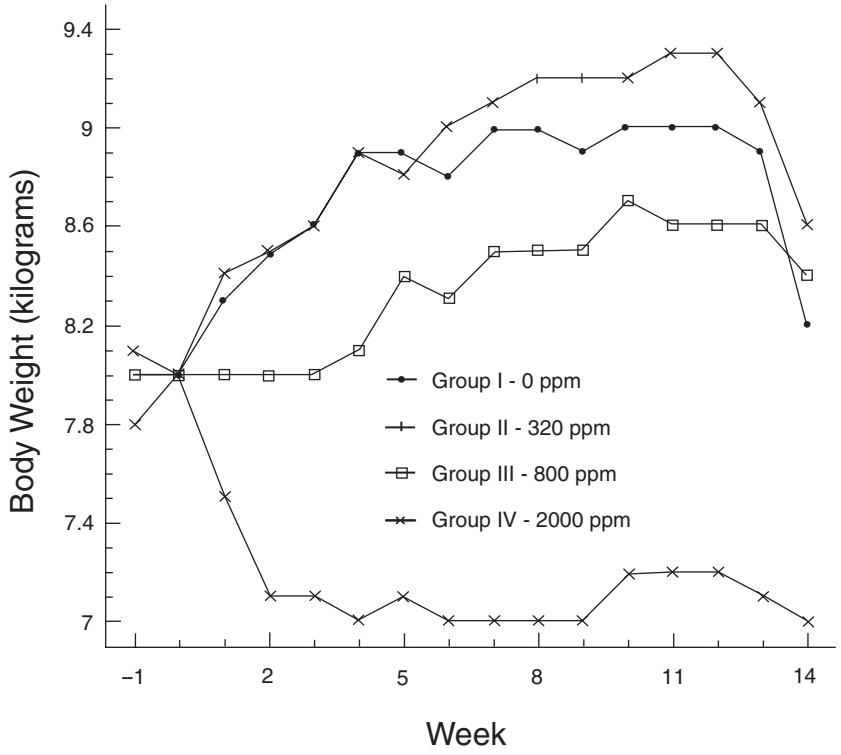
alterations, is not clear. Based on all other evaluations, the NOAEL for dietary administration of acetamiprid to dogs for 3 months under the conditions of this study was 800 ppm (equal to 32 mg/kg bw per day), based on the decrease in body weight at 2000 ppm (equal to 58 mg/kg bw per day).

The study complied with GLP, and a QA statement was attached (Auletta, 1994a).

In the final dog study, acetamiprid (lot No. NNI-03, purity > 99%) was administered orally via dietary admixture to 24 Beagle dogs (four of each sex per group) at a dose level of 240, 600 or 1500 ppm (equal to 9, 20 and 55 mg/kg bw per day for males and 9, 21 and 61 mg/kg bw per day for females, respectively) for a period of at least 12 months. Control animals (four of each sex per group) received untreated standard laboratory diet. At the initiation of the study, the animals were approximately 6 months old, and the mean weights were 9.3 kg (range 7.6–10.3 kg) for males and 8.3 kg (range 7.6–10.1 kg) for females.

Physical observations, ophthalmoscopic examinations, body weight and feed consumption measurements, haematology, biochemistry and urinalyses were performed on all animals pretest and at selected intervals during the treatment period. After at least 12 months of treatment, all animals were sacrificed, selected organs were weighed and organ to body weight and organ to brain weight ratios were calculated. Complete gross postmortem examinations and histopathological evaluation of selected tissues were conducted on all animals.

Figure 5. Group mean body weight values in female dogs



Source: Auletta (1994a)

All animals survived throughout the study. No significant abnormalities were noted during weekly examinations, and daily observations were generally unremarkable.

Terminal ophthalmological examinations revealed no evidence of acetamiprid-related changes.

Moderate mean weight losses (0.5 kg) occurred in high-dose (1500 ppm) females during weeks 1 and 2 and in high-dose males between weeks 2 and 3.

Body weights stabilized after these initial decreases, although mean weights remained lower than concurrent control values for high-dose males and females for the remainder of the study. Mean weights at study termination for high-dose males and females were 16% and 20%, respectively, lower than control values.

Mean body weights and body weight gains for low-dose (240 ppm) and mid-dose (600 ppm) males and females were generally comparable to or higher than control values throughout the study. The initial weight losses seen in high-dose males and females correlated with statistically significant decreases in feed consumption, relative to control values, at weeks 1 and 2. Feed consumption values after week 3 were close to control values for test material–treated males and comparable to or slightly lower than control values for test material–treated females.

Test material intake, based on feed consumption data and nominal dietary concentrations, ranged during the study as shown in Table 26.

Haematology, blood biochemistry and urinalysis evaluations performed periodically throughout the study revealed no evidence of toxicity associated with acetamiprid administration.

No adverse effects of acetamiprid administration on organ weights at study termination were evident.

All the animals survived the experiment and were killed at the end of 12 months. Few gross lesions were observed at the terminal necropsy. The gross lesions occurred sporadically among the

Table 26. Mean test material intake

Group	Dietary concentration (ppm)	Mean test material intake (mg/kg bw per day)			
		Males		Females	
		Range	Average	Range	Average
II	240	8–10	9	7–11	9
III	600	18–23	20	17–25	21
IV	1500	39–65	55	36–75	61

From Auletta (1994b)

Table 27. Comparative NOAELs and LOAELs of three dog studies

Period of study	NOAEL		LOAEL		Critical end-points
	ppm	mg/kg bw per day	ppm	mg/kg bw per day	
28 days	1000	22–34	3000	21–81	Decrease in body weight (11–19% of pretest weight)
3 months	800	32	2000	58	Decrease in body weight
12 months	600	20	1500	55	Initial body weight losses and decreased body weight gains during the study in males and females

control and the treated groups. There were no compound-related lesions among the males and females in this study. There were no compound-related microscopic changes among males and females of this study. The microscopic findings either occurred sporadically or otherwise showed similar incidence between the control and the treated groups.

Based on the initial body weight losses and decreased body weight gains during the study in males and females receiving the highest dietary concentration (1500 ppm, equal to 55 mg/kg bw per day) of acetamiprid, the NOAEL for dietary administration of this material to dogs for 1 year under the conditions of this study was 600 ppm (equal to 20 mg/kg bw per day).

The study complied with GLP, and a QA statement was attached (Auletta, 1994b).

When all three dog studies (28 days, 90 days and 1 year) described above are considered together, it is observed that the common critical toxicological effect is growth retardation, represented by a decrease in body weight. In view of this, there is a need for prescription of an overall NOAEL, which is set at 800 ppm (equal to 32 mg/kg bw per day). Table 27 compares the NOAELs and lowest-observed-adverse-effect levels (LOAELs) of these three dog studies.

(b) Dermal application

Rabbits

Acetamiprid (batch No. NFG-02, purity 99.9%) was evaluated for dermal irritation and systemic toxicity when applied to the dorsal intact skin of male and female New Zealand White rabbits (Hra:[NZW] SPF) (five of each sex per group) 5 days/week for at least 3 consecutive weeks. Dose levels were 100, 500 and 1000 mg/kg bw per day. A fourth group of five male and five female rabbits served as the control and was sham treated. The body weight of animals at the initiation of the study was approximately 2–3 kg.

Survival, clinical signs, body weight and feed consumption data, gross signs of dermal irritation (graded according to Draize), clinical pathology studies (haematology and serum chemistry),

absolute and relative (to body weight) organ weight data, and necropsy and microscopic pathological findings were evaluated.

All animals survived until study termination. Clinical observations, body weight data and feed consumption measurements provided no evidence of toxicity. No signs of compound-induced dermal irritation were observed. There was no evidence of an effect of administration of the test material in the haematology, serum chemistry, necropsy or organ weight data. No compound-related histomorphological findings were observed in tissues that were examined microscopically (liver, kidney and treated and untreated skin).

In conclusion, under the conditions of this study, dermal application of acetamiprid at dose levels of 100, 500 and 1000 mg/kg bw per day for at least 3 consecutive weeks to rabbits of both sexes did not cause systemic toxicity, dermal irritation or histomorphological lesions in any of the tissues examined. In view of the above, the no-observed-effect level (NOEL) is 1000 mg/kg bw per day, the highest dose tested.

The study complied with GLP, and a QA statement was attached (Trutter, 1997).

2.3 Long-term studies of toxicity and carcinogenicity

Long-term studies of oral toxicity in mice and rats were conducted using acetamiprid.

Mice

In an 18-month oncogenicity study, acetamiprid (lot No. NNI-01, purity 99.38%) was offered to 60 male and 60 female Crl:CD-I (ICR)BR mice in the diet at a dose level of 130, 400 or 1200 ppm. The control group (60 animals of each sex) received untreated diet as obtained from the supplier. At the initiation of the study, the mice were 4 weeks old and weighed 23–28 g (males at randomization) and 19–26 g (females at randomization).

Each mouse was observed 3 times daily (twice daily on weekends and holidays) for mortality and overt signs of toxicity. Detailed observations were performed at least once weekly. Individual body weights and feed consumption values were recorded weekly for the first 16 weeks of the study and once every 4 weeks thereafter, and compound consumption was calculated from these values. Feed efficiency was calculated weekly for the first 16 weeks of the study. Average feed consumption and compound consumption are shown in Table 28.

Haematological evaluations were conducted on 10 mice of each sex per group at 12 and 18 months of the study. At the termination of the study, all mice were euthanized. A thorough post-mortem examination was conducted, and selected organ weights were determined at the interim and terminal sacrifices. Protocol-specified tissues were processed histologically, and a microscopic examination was conducted. Photomicrographs of representative lesions seen in microscopy were taken. A peer review was performed on all target organs and neoplasms and on all tissues from 10% of the mice randomly selected from the control and 1200 ppm groups.

No test article effect on survival was noted. Decreased defecation, decreased body weight and decreased feed consumption were observed in males and females at the 1200 ppm dose level. Body weights were slightly decreased in males at 400 ppm, and statistical significance was attained sporadically. The body weight gain of males at 400 ppm was statistically significantly decreased compared with controls through 13 weeks of study but was comparable to that of controls during the remainder of the study. In both males and females at 130 ppm, feed consumption was slightly increased over controls. No differences between the groups were noted for the haematological parameters. There were no treatment-related observations at necropsy, but at the end of 18 months, mean liver to body weight ratios were increased in males and females receiving 1200 ppm and also in females receiving 400 ppm. On microscopic examination, treatment-related hepatocellular hypertrophy was seen

Table 28. Average feed consumption and compound consumption

Dietary concentration (ppm)	Feed consumption (g/kg bw per day)		Compound consumption (mg/kg bw per day)	
	Males (%)[a]	Females (%)	Males	Females
0 (control)	157 (NA)	185 (NA)	NA	NA
130	156 (−0.6)	194 (+4.9)	20.3	25.2
400	164 (+4.4)	190 (+2.7)	65.6	75.9
1200	155 (−1.3)	179 (−3.2)	186.3	214.6

From Goldenthal (1994)
NA, not applicable
[a] Per cent difference from control.

in male and female mice receiving 1200 ppm at the 12-month interim sacrifice and at 18 months. An extremely low incidence of hepatocellular hypertrophy was present in males and females at 400 ppm. Hepatocellular hypertrophy is generally considered to be a pharmacologically adaptive response of the liver to exposure to a xenobiotic.

The only other statistically significant finding was an increased incidence of myeloid hyperplasia in the bone marrow of the femur in 1200 ppm males euthanized at the interim sacrifice. This change was considered spurious, as the increase was small, it was not significant when both mice at interim sacrifice and mice that died or were sacrificed in extremis were considered together, and the incidence in the bone marrow of the sternum was not statistically significantly increased.

There were no other test article–related effects noted.

Statistical analysis of tumour incidence showed no positive trends or differences.

Based on transient decreased body weight observed at 400 ppm (equal to 65.6 mg/kg bw per day) in males, the NOAEL of acetamiprid was considered to be 130 ppm (equal to 20.3 mg/kg bw per day). There was no evidence of an oncogenic effect in mice.

The study complied with GLP, and a QA statement was attached (Goldenthal,1994; Cunny, 2000a; Gopinath, 2001).

Rats

To evaluate the oncogenic potential as well as the general toxicity of acetamiprid, acetamiprid (lot No. NN I-01, purity 99.7%) was offered to groups of Crl: CD BR rats (60 of each sex per group) in the diet at a concentration of 160, 400 or 1000 ppm (equal to 7.1, 17.5 and 46.4 mg/kg bw per day for males and 8.8, 22.6 and 60 mg/kg bw per day for females, respectively) for 2 years. The control group (60 rats of each sex) was given untreated diet as obtained from the supplier. At the initiation of the study, the rats were 6 weeks old and weighed 164–208 g and 137–157 g for males and females, respectively, at randomization. Fresh diet was offered weekly.

Each rat was observed twice daily for mortality and signs of toxicity. Detailed clinical examinations were conducted weekly. Individual body weights and feed consumption were determined weekly for the first 14 weeks of the study and every 2 weeks thereafter. Feed efficiency was calculated weekly for the first 14 weeks of the study. An ophthalmoscopic examination was performed on all rats pretest and at 6, 12 and 24 months. Clinical pathology studies were conducted on 10 randomly selected male and female rats from each group at 3, 6, 12, 18 and 24 months, and, when possible, the same rats were used at each time interval. Complete necropsy examinations were conducted on rats that died on study or were euthanized in extremis, on 10 rats of each sex per group at the 12-month interim sacrifice and on all surviving rats at study termination. Selected organ weights were determined at the interim and terminal sacrifices. Protocol-specified tissues were processed histologically, and microscopic examinations were conducted and peer-reviewed.

Increased incidences of clinical signs were observed in males, such as rales, hunched posture, laboured breathing and red/brown material around the nose; hunched posture and laboured breathing were also observed in the females, particularly in the latter part of the study. These increased incidences occurred mainly in the 400 and 1000 ppm groups. Other in-life observations included low weekly/biweekly mean body weights and body weight gains and high mean feed consumption values (1000 ppm males; 400 and 1000 ppm females), increased mean water consumption secondary to high feed consumption for the 1000 ppm males (week 24) and females (week 76) and, possibly, low mean serum triglyceride concentrations for the 1000 ppm females.

Average feed and compound consumption values (weeks 1–104) are summarized in Table 29.

Table 30 summarizes the body weight data at study termination (104 weeks).

There were no apparent test article–related changes in haematological parameters. As regards the serum biochemical values, at 12 and 18 months and at study termination, females in the 1000 ppm group had lower triglyceride values than controls. These differences were statistically significant at 12 and 18 months and were considered to be possible effects of acetamiprid administration. These changes may be secondary to altered lipid metabolism, as the rats had higher feed consumption, but lower body weights, than controls. Some biochemical parameters were occasionally statistically significantly different from control values; however, these were considered incidental and not associated with test article administration.

Test article–related pathological changes included trace to mild centrilobular hepatocellular hypertrophy in the 400 and 1000 ppm males and the 1000 ppm females; trace to moderate hepatocellular vacuolation in the 400 and 1000 ppm males; and trace to severe microconcretions (calculi) in the renal papillae of the 1000 ppm males (Tables 31 and 32).

The incidences of hepatocellular hypertrophy and hepatocellular vacuolation were statistically significantly increased in the 400 and 1000 ppm male treatment groups when only the animals of the 12 month to termination study interval were analysed (Table 32), and also when the combined incidences (all animals from both study intervals) were analysed. Both findings were considered to be test article related in males in these treatment groups. In females, the incidence of hepatocellular hypertrophy was statistically significant ($P \leq 0.01$) only when all animals from both sacrifice intervals were combined and only in the 1000 ppm treatment group; hepatocellular hypertrophy was also considered test article related in the 1000 ppm female treatment group. The incidence of hepatocellular vacuolation did not achieve statistical significance in any female treatment group, regardless of the manner in which the animals were grouped for analysis, and was therefore not considered a test article–related effect in this sex. There were no macroscopic observations or organ weight changes that correlated with these findings.

At the time of the terminal sacrifice, compound-related non-proliferative microscopic changes were identified in the kidney of the 1000 ppm group males. Trace to severe microconcretions of the renal papilla were seen with increased incidence and severity in the 1000 ppm group males and were considered to be compound related in that treatment group (Table 33).

The incidence of microconcretions in all male groups (range 35.4–75.5%) exceeded MPI Research historical control values (range 0–14.82%; calculus/calculi used synonymously with microconcretions) and was statistically significantly increased ($P \leq 0.01$) in the 1000 ppm group when the animals of the 12 months to termination study interval were analysed, and also when the combined incidence (all animals from both sacrifice intervals) was analysed. Microconcretions of the renal papilla were not increased significantly in incidence or severity in females. There were no compound-related macroscopic or organ weight findings that correlated with these microscopic observations.

There were no proliferative lesions in any treatment group of either sex that were considered to be definitively test article related. In females, there was an increased incidence of hyperplasia of the mammary gland observed in the 1000 ppm group, which attained statistical significance. The incidences of these findings are presented in Table 34.

Table 29. Average feed consumption and compound consumption

Dietary concentration (ppm)	Feed consumption (g/kg bw per day)		Compound consumption (mg/kg bw per day)	
	Male (%)[a]	Female (%)[a]	Male	Female
0 (control)	43.9 (NA)	55.3 (NA)	NA	NA
160	44.3 (+0.9)	55.3 (−0.4)	7.1	8.8
400	43.9 (0.0)	56.5 (+2.2)	17.5	22.6
1000	46.4 (+5.7)	60.0 (+8.5)	46.4	60.0

From Hatch (1994)
NA, not applicable
[a] Per cent difference from control.

Table 30. Body weight at study termination (104 weeks)

Dietary concentration (ppm)	Body weight			
	Males		Females	
	g	%[a]	g	%[a]
0 (control)	667	NA	433	NA
160	668	+0.1	453	+4.6
400	673	+0.9	388	−10.4
1000	578	−13.3	367	−15.2

From Hatch (1994)
NA, not applicable
[a] Per cent difference from control.

Table 31. Test article–related observations (0–12 months interim)

	Dietary concentration (ppm)			
	0 (control)	160	400	1000
Number of animals examined	*10*	*11*	*13*	*11*
Males				
Liver hypertrophy	0	0	5*	10**
- trace	0	0	5	8
- mild	0	0	0	2
Hepatocyte vacuolation	2	4	10 **	10**
- trace	2	3	9	8
- mild	0	0	1	2
- moderate	0	1	0	0
Females				
Liver hypertrophy	0	0	0	4
- trace	0	0	0	4 *

From Hatch (1994)
* $P \leq 0.05$; ** $P \leq 0.01$

Table 32. Test article–related observations in males (12 months to termination)[a]

	Dietary concentration (ppm)			
	0 (control)	160	400	1000
Number of animals examined	*48*	*50*	*48*	*49*
Liver hypertrophy	0	0	15**	34**
- trace	0	0	15	22
- mild	0	0	0	12
Hepatocyte vacuolation	10	9	22*	29**
- trace	7	6	17	18
- mild	2	3	5	10
- moderate	0	0	0	1
- severe	1	0	0	0

From Hatch (1994)
* $P \leq 0.05$; ** $P \leq 0.01$
[a] The changes were not observed in females.

Table 33. Compound-related observations in male kidney, 12 months to termination

	Dietary concentration (ppm)			
	0 (control)	160	400	1000
Number of animals examined	*48*	*50*	*48*	*49*
Microconcretion of papilla	17	23	23	37*
- trace	16	21	17	26
- mild	1	2	4	8
- moderate	0	0	2	2
- severe	0	0	0	1

From Hatch (1994)
* $P \leq 0.01$

However, the severity of mammary gland hyperplasia was trace to mild in most cases, and there was little, if any, significant increase in severity in a dose-related manner. No increase in incidence or severity of this finding was evident in any female treatment group at the 12-month interim necropsy interval. When all females on study were analysed as a group, the incidence of hyperplasia in the 1000 ppm group (26/60; 43.3%) did not exceed the range of historical control values for this finding at MPI Research (range 0–58.6%), and in no test article treatment group did the incidence of mammary gland hyperplasia attain statistical significance when compared with the incidence in the control group.

Because of the above considerations, the toxicological significance of mammary hyperplasia in the 1000 ppm treatment group is uncertain.

Although the incidence of mammary gland adenocarcinoma was statistically significant ($P \leq 0.05$) when analysed by the Cochran Armitage Trend Test and Peto Test, adenocarcinomas of the mammary gland were not considered to be a test article–related finding. Historical control data maintained at MPI Research show a range of incidences for mammary gland adenocarcinoma from 13.3% to 28.6%; the incidence of mammary gland adenocarcinoma in 400 ppm females of this study (16/60; 26.6.3%) and in 1000 ppm females of this study (17/60; 28.3%) is within the range of historical values, albeit just barely. In no case was the incidence of mammary gland adenocarcinoma in

Table 34. Incidence of hyperplasia/neoplasia of the mammary gland (all female animals combined)

	Dietary concentration (ppm)			
	0 (control)	160	400	1000
Number of animals examined	*59*	*60*	*60*	*60*
Mammary gland				
Hyperplasia	17	13	16	26*
- trace	12	10	12	19
- mild	5	3	3	6
- moderate	0	0	1	1
Fibroadenoma	17	15	10	15
Adenoma	1	0	4	3
Benign tumour (adenoma/fibroadenoma)	18	15	14	18
Adenocarcinoma	10	11	16	17**
Any mammary tumour	24	21	24	29

From Hatch (1994)
* $P \leq 0.01$ by Fisher's exact test; ** $P \leq 0.05$ by Cochran Armitage Trend Test and Peto Test

any test article treatment group significantly different from the incidence in the control group when analysed using the Fisher Exact Test. The incidences of animals with any mammary tumours (adenoma, fibroadenoma and/or adenocarcinoma) in the test article–treated group were not substantially different from the control group.

There were no test article–related proliferative lesions or neoplasms observed in this study.

An MPI Research Toxicology Working Group was convened to examine the increased incidences of certain clinical signs, particularly rales in the 160 ppm males. The Toxicology Working Group concluded that rales were not test article related in this group. As rales were the only potentially dose-related observation made in the 160 ppm group, the NOAEL of acetamiprid in this study was 160 ppm (equal to 7.1 mg/kg bw per day), based on trace centrilobular liver hypertrophy and hepatocyte vacuolation at 400 ppm (equal to 17.5 mg/kg bw per day).

Acetamiprid was not oncogenic in rats.

The study complied with GLP, and a QA statement was attached (Hatch, 1994; Pallen, 1999; Cunny, 2000b).

2.4 Genotoxicity

Acetamiprid was tested for genotoxicity in nine genotoxicity studies, of which six were in vitro studies and three were in vivo studies (Table 35). Acetamiprid gave negative results in all in vivo studies and four in vitro studies. In studies of chromosomal aberrations (mouse lymphoma forward mutation assay) in vitro, although acetamiprid was found to be negative without metabolic activation, it was questionable for inducing forward mutation with metabolic activation. In another study of chromosomal aberrations (in Chinese hamster ovary cells) in vitro, acetamiprid induced chromosomal aberration with metabolic activation and weakly without metabolic activation.

All the studies complied with GLP, and QA statements were attached.

On the basis of these studies, it is concluded that acetamiprid is unlikely to be genotoxic.

Table 35. Results of genotoxicity studies with acetamiprid

Genotoxicity study	Strain/species	Concentration	Purity (%)	Result	Reference
In vitro					
Reverse mutation study	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 *Escherichia coli* WP2*uvrA*	313, 625, 1250, 2500, 5000 µg/plate	99.2	Negative with S9 mix Negative without S9 mix	Kanaguchi (1993a)
DNA repair test on bacteria (rec assay)	*Bacillus subtilis* M45(rec−) and H17(rec+)	1359, 2718, 5435, 10 870, 21 740 µg/disc without metabolic activation, 679.4, 1359, 2718, 5435, 10 870 µg/disc with metabolic activation	99.46	Negative	Kanaguchi (1992b)
Gene mutation, mammalian cells[a]	CHO cells (*HPRT* locus)	*Without S9 mix* Test 1: 500, 1000, 2000, 2500, 3000, 3500, 4000 µg/ml Test 2: 1000, 2000, 2500, 3000, 3500, 4000 µg/ml *With S9 mix* Test 1: 250, 500, 1000, 1500, 2000, 3000, 3500, 4000 µg/ml Test 2: 500, 1000, 1500, 2000, 2250, 2500, 2750 µg/ml	Not given	Negative	Adams (1997)
Gene mutation/chromosomal aberration, mammalian cells[b]	Mouse lymphoma forward mutation assay, L5178Y Tk+/− cells (*TK* locus)	63.5–2000 µg/ml	99.57	Negative without metabolic activation Questionable for inducing forward mutation with metabolic activation	Cifone (1994)
Chromosomal aberration, mammalian cells[c]	CHO cells	17.0, 33.9, 67.8, 135.6, 271.3, 542.5, 1085, 2170 µg/ml	99.2	Induced chromosomal aberration with metabolic activation and weakly without metabolic activation	Kanaguchi (1992a)
Unscheduled DNA synthesis[d]	Rat primary hepatocytes	10.1–505 µg/ml	99.57	Negative	Ham (1994)

Table 35 (continued)

Genotoxicity study	Strain/species	Concentration	Purity (%)	Result	Reference
In vivo					
Micronucleus test[e]	CD-1 (ICR) mouse	Oral (gavage, one application) 20, 40, 80 mg/kg bw	99.57	Negative	Murli (1994a)
Chromosomal aberration test (bone marrow cells)[f]	Sprague-Dawley CD strain rat	Single oral dose of 250 mg/kg bw (gavage)	99.46	Non-clastogenic	Durward (1993)
UDS[g]	Sprague-Dawley strain rat (liver cell)	75, 150, 300 mg/kg bw	99.9	Negative	San & Sly (1997)

CHO, Chinese hamster ovary; DNA, deoxyribonucleic acid; HPRT, hypoxanthine-guanine phosphoribosyl transferase; S9, 9000 × *g* rat liver supernatant; TK, thymidine kinase; UDS, unscheduled DNA synthesis

[a] Acetamiprid was tested for its ability to induce forward mutation at the functionally hemizygous *HPRT* locus in CHO cells in vitro in both the presence and the absence of exogenous metabolic activation in the form of Aroclor 1254–induced rat liver S9. Toxicity was observed after treatment with acetamiprid in all of the tests, in both the absence and the presence of S9 mix. No significant increases in mutant frequency were observed in cultures treated with acetamiprid in any of the tests in either the absence or the presence of S9 mix. The positive controls induced highly significant increases in mutant frequency in all of the tests in both the absence and the presence of S9 mix. It was concluded that acetamiprid did not demonstrate mutagenic potential in this in vitro mammalian cell gene mutation assay.

[b] The assay was to evaluate the ability of acetamiprid to induce forward mutations at the *TK* locus in the L5178Y mouse lymphoma cell line. The test material was soluble in dimethyl sulfoxide at 500 mg/ml. In the preliminary cytotoxicity assay, cells were exposed to the test material at concentrations from 1.95 to 1000 µg/ml for 4 hours in the presence and absence of rat liver S9 metabolic activation. The test material remained in solution in culture medium at all concentrations tested. The test material was weakly to moderately cytotoxic with and without metabolic activation at 1000 µg/ml, and lower concentrations were non-toxic. The mutation assays were initiated with treatments up to about 5070 µg/ml in an attempt to obtain more cytotoxic dose levels. Four non-activation and four S9 metabolic activation mutation assays were initiated. The studies were repeated several times in an attempt to clarify a response and because shifts in cytotoxicity occurred. The test material produced dose-related increases in toxicity in all mutation trials. In the non-activation trials, the test material was lethal or excessively toxic between 1500 µg/ml and about 3000 µg/ml. Treatments that induced less than 10% relative growth were not used in the analysis, as these results are considered unreliable. None of the remaining treatments induced mutant frequencies that exceeded the minimum criterion for a positive response. In the presence of metabolic activation, treatments from 63.5 to about 2000 µg/ml were evaluated. Small increases that just exceeded the minimum criterion for a positive response were observed in two trials. One trial did not have highly toxic treatments, and the results were inconclusive. The last trial had one treatment that approached, but did not meet, the minimum criterion for a positive response. The test material was therefore considered to have questionable activity with activation. Acetamiprid was therefore evaluated as negative without metabolic activation and questionable for inducing forward mutations at the *TK* locus in L5178Y mouse lymphoma cells in the presence of S9 metabolic activation and under the conditions used in this study.

[c] In the direct method, chromosomal aberration was slightly increased ($P < 0.05$) at 175 and 700 µg/ml in comparison with that in the solvent control. In the metabolic activation method, chromosomal aberration was significantly increased with a dose–response relationship at the middle and high test concentrations (675 and 1350 µg/ml). The frequencies of chromosomal aberration did not increase in the metabolic activation without S9 mix (reference test). Benzo(*a*)pyrene, which requires metabolic activation for expression of mutagenicity, increased chromosomal aberrations in the presence of S9 mix, but not in the absence of S9 mix. Through both methods, chromosomal aberration frequencies in the negative and the solvent controls were in the range of historical background data. On the basis of the results, the author considered that acetamiprid induced chromosomal aberration with metabolic activation, and its activity was lower without metabolic activation.

[d] In the assay for UDS in rat liver primary cell cultures, the test material, acetamiprid, did not induce significant increases in UDS in two independent trials. In each trial described in Ham (1994), freshly prepared rat hepatocytes were exposed to acetamiprid at concentrations ranging from 5000 to 0.500 µg/ml in the presence of 0.37 MBq/ml ^{3}H-methyl thymidine (1554 GBq/mmol). In Trial 1, 15 treatments from 5000 to 0.500 µg/ml were initiated. The test material was insoluble in media at concentrations of 5000 and 4000 µg/ml, with apparent solubility occurring at 3000 µg/ml. Five treatments from 5000 to 1000 µg/ml were not analysed for nuclear labelling as a result of high toxicity. Six treatments from 500 to 10.0 µg/ml covered a good range of toxicity (53.2–98.4% survival) and were selected for analysis of nuclear labelling. None of the criteria used to indicate UDS were approached by the chemical treatments in Trial 1, and no dose-related response was observed. A second trial was initiated to confirm these results. Based upon cytotoxicity information obtained in Trial 1, 12 dose levels from 2020 to 0.505 µg/ml were initiated in Trial 2. Treatments from 2020 to 1010 µg/ml were not analysed as a result of high toxicity. Six treatments from 505 to 10.1 µg/ml covered a good range of toxicity (64.4–107.5% survival) and were selected for analysis of nuclear labelling. None of the criteria used to indicate UDS were approached by the chemical treatments in Trial 2. The data confirmed the results from Trial 1, and acetamiprid was evaluated as inactive in both trials of the rat primary hepatocyte UDS assay.

[e] In this mouse micronucleus assay, animals were dosed at 20, 40 and 80 mg/kg bw. Ten animals (5 males and 5 females) were randomly assigned to each dose/harvest time group. Positive control groups euthanized approximately 24 hours after dosing were included in the assay. The animals dosed with the test article and the vehicle control were euthanized approximately 24, 48 and 72 hours after dosing for extraction of bone marrow. The test material, acetamiprid, did not induce a significant increase in micronuclei in bone marrow polychromatic erythrocytes under the conditions of this assay.

[f] The study was performed to assess the potential of acetamiprid to produce damage to chromosomes or the mitotic apparatus of rats when administered by the oral route. The method used followed that described in the OECD guidelines. Following a preliminary range-finding study to confirm the oral toxicity of the test material, the chromosomal aberration study was conducted using acetamiprid at the maximum tolerated dose of 250 mg/kg bw. In the study, groups of 10 rats (5 males and 5 females) were given a single oral dose of acetamiprid at the maximum tolerated dose (250 mg/kg bw). Animals were killed 6, 24 or 48 hours later, the bone marrow was extracted and slide preparations were made and stained. Bone marrow cells were scored for the presence of chromosomal aberrations. Further groups of rats were treated with arachis oil BP or cyclophosphamide, to serve as vehicle and positive controls, respectively. There was no evidence of an increase in the incidence of chromosomal aberrations in animals treated with acetamiprid when compared with the vehicle control groups. The positive control material produced a marked increase in the frequency of chromosomal aberrations. Acetamiprid was considered to be non-clastogenic under the conditions of the test.

[g] Tested in the UDS test with mammalian liver cells in vivo. The assay was performed in two phases. The first phase, the initial and repeated dose range–finding assays, was a toxicity study used to aid in the selection of dose levels to be used in the UDS assay. The second phase, the UDS assay, was used to evaluate the potential of the test article to induce UDS in hepatocytes of exposed male rats. In all phases of the study, test and control articles were administered at a constant volume of 10 ml/kg bw by a single oral gavage injection. In the initial dose range–finding assay, male rats were exposed to 50, 100, 200, 400 or 1250 mg of test article per kilogram body weight. All five dose preparations were prepared independently, vortexed upon preparation and vortexed again immediately before dosing the animals. Weight loss was observed at all dose levels, with only the animals in the 50 and 100 mg/kg bw dose groups regaining body weight on day 3. As the LD_{50} (1640 mg/kg bw) calculated in this study was different from that of the earlier study (217 mg/kg bw), the dose range–finding assay was repeated. In the repeated dose range–finding assay, male rats were exposed to 50, 100, 200, 400 or 1250 mg of test article per kilogram body weight. Weight loss was observed in animals of the 200 and 400 mg/kg bw dose groups, whereas the 100 mg/kg bw animals exhibited weight gain on day 1 and weight loss on day 3. The 50 mg/kg bw animals showed minimal weight gain on days 1 and 3. The 1250 mg/kg bw animals were found dead before a weight determination could be made on day 1. Body weights were determined prior to treatment, on day 1 and day 3. In the UDS assay, male rats were exposed to 75, 150 and 300 mg of test article per kilogram body weight. The selection of 300 mg/kg bw as the high dose was based on the clinical signs and weight loss observed at 400 mg/kg bw and weight loss observed at 200 mg/kg bw (in the repeated dose range–finding assay). In the UDS assay, no mortality was observed in any treated or control rats. Clinical signs were normal in all dose groups following dose administration and prior to harvesting of the hepatocytes after exposures for 2–4 hours and 12–16 hours, with the exception of one animal in the 300 mg/kg bw dose group exposed for 2–4 hours, which exhibited lethargy and tremors. However, this animal was not used in the hepatocyte harvest. Upon perfusion, the livers of the 300 mg/kg bw animals exposed for 2–4 hours and 12–16 hours were observed to be much darker than the livers from any of the other dose groups. The test article, acetamiprid, did not induce a significant increase in the mean number of net nuclear grain counts (i.e. an increase of at least five counts over the negative control group) in hepatocytes isolated either 2–4 hours or 12–16 hours after dose administration. Acetamiprid was concluded to be negative in the UDS test with mammalian liver cells in vivo.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

A range-finding study was undertaken to determine the dose levels to be employed in a multi-generation reproduction study in rats. Charles River Crl:CD VAF/Plus rats were randomly assigned to one control and four treatment groups of 10 animals each (5 males and 5 females). The age of the rats at the time of initiation of the study was 71 days for both males and females. The male rats weighed between 343 and 374 g, and the females weighed between 211 and 239 g.

Acetamiprid (lot No. NNI-01, purity 99.38%) was administered orally via the diet to four groups of rats (males and females) beginning 14 days prior to mating and continuing until euthanasia. Animals in the treatment groups received the test material in the diet at concentrations of 125, 250, 500 and 1000 ppm (average equal to 8.3, 16.6, 31.4 and 59.7 mg/kg bw per day for males and 9.0, 18.0, 33.7 and 63.6 mg/kg bw per day for females, respectively). The control group received only the ground basal laboratory diet on a comparable regimen.

The mated females were allowed to deliver. The litters were evaluated for survival and growth. The dams and their litters were euthanized on lactation day 4.

The death of a single male rat was observed at the 250 ppm level. This death was not considered to be related to treatment, as no signs of overt toxicity were observed prior to death and no other deaths were observed at higher dose levels.

No clinical signs of toxicity or gross necropsy findings were observed for any animal that survived to scheduled euthanasia.

Treatment-related reductions in body weight and feed consumption were observed at dose levels of 500 and 1000 ppm when compared with the control groups. These reductions were observed for males and females. Reductions observed for females during weekly measurements often continued through the gestation and lactation periods as well.

There was no indication of impaired fertility as a result of treatment. Male and female copulatory and fertility indices of the treatment groups were generally comparable with the corresponding control indices. Offspring growth was inhibited (as evidenced by reduced litter weights, relative to the control values) by treatment of the F_0 dams at dose levels of 500 and 1000 ppm. Development of the F_1 offspring was also adversely affected by treatment of the dams, as evidenced by the reduction in the number of implantation sites at the 1000 ppm level when compared with the control group.

The study complied with GLP, and a QA statement was attached (Schardein, 1991).

In a two-generation reproduction study in Charles River Crl:CD VAF/Plus rats, the test article, acetamiprid (lot No. NNI-01, purity 99.38%), was administered orally in the ground diet and was available ad libitum. Dietary concentrations of 100, 280 and 800 ppm were evaluated. The control group received the basal laboratory diet on an identical regimen. Each group (F_0 parents) consisted of 26 males and 26 females; these animals were treated beginning at 37 days of age for 59 days prior to the F_1 mating. The F_0 parents were mated to produce the F_1 litters. From the F_1 litters, 26 males and 26 females were selected to become F_1 parents. These F_1 parental animals were treated beginning at 22 days of age for a minimum of 64 days prior to mating to produce the F_2 generation. Treatment continued through mating, gestation and lactation. The control group received the basal laboratory diet on the same regimen.

The F_0 animals were approximately 14 weeks old at the time of pairing; males and females weighed 137–158 g and 125–152 g, respectively, at randomization on the day prior to the 1st day of treatment.

The average acetamiprid consumptions of the F_0 animals are shown in Table 36.

The average acetamiprid consumptions of the F_1 animals are shown in Table 37.

All parental animals and offspring were observed for mortality and overt toxicity twice each day, 7 days/week. Detailed clinical observations of the adults were recorded at least once weekly. Body weights for the parental males were recorded weekly, as was feed consumption, except during the mating periods. Body weights for the females were recorded weekly until evidence of copulation; feed consumption was recorded weekly until the initiation of the mating period. Maternal body weights and feed consumption were recorded at specified intervals during gestation and lactation. The F_0 and F_1 parental reproductive performance was assessed, and the F_1 and F_2 litters were evaluated for viability and growth.

All parental animals and offspring were subjected to gross necropsy at scheduled sacrifice or time of death. Selected organs from the parental animals were preserved, and the tissues were examined microscopically. The weights of the testes were recorded, and an evaluation of spermatogenesis was conducted at necropsy for any adult male that failed to sire a litter.

At the high dose level (800 ppm), statistically significant decreases in weekly body weights relative to the control values were noted throughout the study for the males in the F_0 generation (Table 38), but not for females (Table 39). Significantly reduced maternal body weight gain compared with that of the control group occurred early in the gestation period for the F_0 females in this treated group, and some reduction of body weight was present thereafter (Tables 40 and 41).

The mean maternal body weight gains for the F_0 high-dose females were also reduced relative to those of the control group during the lactation period (Tables 42 and 43).

Similarly, significant inhibition of growth of F_1 offspring in comparison with the control values occurred at this dose level, and depressed body weights continued to be evident for the F_1 adults (Tables 44 and 45). Significantly decreased body weight and body weight gain were also seen in the high-dose group in comparison with the control group of the F_1 generation during gestation and lactation (Tables 46–49).

Reductions in F_2 offspring body weights relative to the control values were also observed in the high-dose group (Table 50).

In both the F_0 and F_1 generations, there were reductions in parental feed consumption values during the first few weeks of the study for males and females in the high-dose group in comparison with the control values. Decreased feed consumption was also evident for the high-dose dams in both generations during gestation and lactation. At the intermediate dose level (280 ppm), reductions in F_0 parental feed consumption were noted in the first 2 weeks of the study and during gestation, but the difference from the control group data was less pronounced than that at the high dose level.

At the low dose level (100 ppm), statistically significant reductions in feed consumption were noted for the F_0 males in the first 2 weeks of the study. These reductions, as well as those seen at other treatment levels during these weeks, may have been due to the rats initially finding the diet unpalatable. Slight reductions in feed consumption during the gestation period were also evident for the F_1 females in the low-dose group but were not considered adverse, as they did not occur in the premating or lactational periods and did not result in statistically significantly reduced body weights. These variations in feed consumption did not represent significant toxicity (Tables 51–54).

Treatment-related microscopic changes in the liver consisting of hepatocyte hypertrophy (males and females, F_0 and F_1 generations) and vacuolar change in hepatocytes (F_1 males) were noted in the high-dose group.

No adverse effects on F_1 offspring survival were observed in the high-dose group. However, biologically and/or statistically significant reductions in the F_2 offspring survival indices at lactation days 0 and 4 were observed in the high-dose litters in comparison with the control values. The

Table 36. Average acetamiprid consumption by F_0 animals

Dietary concentration (ppm)	Average F_0 acetamiprid consumption (mg/kg bw per day)		
	Males (weekly)	Females (weekly)	Females (gestation)
0	0	0	0
100	6.67	8.42	5.04
280	18.9	23.1	13.9
800	54.6	66.5	38.7

From York (1994)

Table 37. Average acetamiprid consumption by F_1 animals

Dietary concentration (ppm)	Average F_1 acetamiprid consumption (mg/kg bw per day)		
	Males (weekly)	Females (weekly)	Females (gestation)
0	0	0	0
100	7.60	9.40	5.67
280	23.3	27.0	15.1
800	65.0	87.1	45.7

From York (1994)

Table 38. Males: Summary of body weight values, F_0 weekly

Week of study	Body weight (g)											
	0 ppm (control)			100 ppm			280 ppm			800 ppm		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
0	148	4.6	26	147	5.4	26	149	5.5	26	145	5.1	26
1	211	5.9	26	208	8.1	26	211	9.4	26	194*	7.5	26
2	259	8.7	26	255	11.2	26	259	16.0	26	239**	11.9	26
3	301	12.9	26	299	16.2	26	303	17.1	26	280**	15.5	26
4	330	16.3	26	328	19.7	26	333	21.6	26	309**	18.6	26
5	359	19.5	26	359	23.3	26	363	24.6	26	335**	20.6	26
6	381	24.7	26	382	26.4	26	388	26.8	26	359**	22.8	26
7	405	26.5	26	406	30.5	26	413	30.1	26	381**	22.6	26
8	422	31.3	26	417	28.3	26	428	29.9	26	394**	23.4	26
9	425	31.9	26	425	29.4	26	439	33.7	26	401**	22.9	26
10	445	33.9	26	443	30.9	26	458	35.8	26	420*	24.2	26
11	462	36.7	26	463	33.8	26	476	36.6	26	437*	24.7	26
12	468	35.9	26	469	37.9	26	478	36.5	26	440*	25.3	26
13	484	40.8	26	487	38.6	26	494	40.8	26	453*	32.1	26
14	485	42.9	26	486	37.3	26	498	42.0	26	455*	35.3	26
15	500	42.6	26	500	37.3	26	512	44.2	26	467*	35.1	26
16	508	43.8	26	507	38.0	26	517	46.1	26	472**	37.2	26
17	512	44.8	26	516	40.0	26	524	48.0	26	483*	35.5	26
18	521	44.5	26	523	40.4	26	534	49.4	26	488*	39.9	26

From York (1994)
N, number of animals; SD, standard deviation; * $P < 0.005$; ** $P < 0.001$

Table 39. Females: Summary of body weight values, F_0 weekly[a]

Week of study	Body weight (g)											
	0 ppm (control)			100 ppm			280 ppm			800 ppm		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	N
0	141	7.9	26	140	6.8	26	139	7.8	26	142	7.3	26
1	181	10.9	26	183	8.8	26	179	7.8	26	177	8.7	26
2	206	14.2	26	205	10.9	26	200	9.2	26	200	11.0	26
3	227	17.7	26	230	15.5	26	223	11.5	26	219	14.8	26
4	241	17.5	26	244	15.2	26	238	13.1	26	233	13.5	26
5	256	19.9	26	266	17.5	26	256	12.8	26	247	13.5	26
6	268	19.6	26	275	18.3	26	265	15.9	26	259	14.6	26
7	282	22.0	26	289	21.9	26	278	15.8	26	272	17.5	26
8	286	22.5	26	295	22.2	26	284	17.4	26	277	18.1	26
9	314	18.6	4	311	22.6	6	306	11.7	3	300	9.5	3
10	345	19.1	2	329	16.3	2	299	NA	1	318	NA	1
11	376	NA	1	363	NA	1	292	NA	1	NA	NA	0
12	448	NA	1	400	NA	1	297	NA	1	NA	NA	0
13	NA	NA	0	NA	NA	0	297	NA	1	NA	NA	0
14	NA	NA	0	NA	NA	0	301	NA	1	NA	NA	0
15	NA	NA	0	NA	NA	0	278	NA	1	NA	NA	0

From York (1994)
N, number of animals; NA, not applicable; SD, standard deviation
[a] No statistical significance observed.

Table 40. Females: Summary of body weight values, F_0 gestation

Days	Body weight (g)											
	0 ppm (control)			100 ppm			280 ppm			800 ppm		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
0	293	23.3	25	299	21.1	22	289	21.9	20	281	18.2	21
6	329	24.2	25	332	24.1	25	320	22.9	25	306**	18.8	25
15	369	24.9	25	373	30.4	25	357	31.2	25	344**	27.2	25
20	436	39.7	25	438	48.3	25	420	55.9	25	404	50.4	25

From York (1994)
N, number of animals; SD, standard deviation; ** $P < 0.001$

Table 41. Females: Summary of body weight changes, F_0 gestation

Days	Body weight changes (g)											
	0 ppm (control)			100 ppm			280 ppm			800 ppm		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
0–6	36	10.0	25	34	9.4	22	33	5.9	20	27**	7.4	21
6–15	40	12.7	25	41	16.0	25	38	20.9	25	38	17.4	25
15–20	67	21.0	25	65	26.3	25	63	30.5	25	60	27.5	25
0–20	143	32.5	25	154	21.9	25	156	15.6	20	143	14.4	21

From York (1994)
N, number of animals; SD, standard deviation; ** $P < 0.001$

Table 42. Females: Summary of body weight values, F_0 lactation

Day of lactation	Body weight (g)											
	0 ppm (control)			100 ppm			280 ppm			800 ppm		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
0	342	25.9	23	347	21.1	23	338	20.2	20	326*	15.7	21
7	360	20.0	24	361	19.9	23	354	29.3	20	340*	21.8	21
14	387	22.6	24	384	23.5	23	376	28.8	20	355**	20.0	21
21	357	22.2	24	348	22.2	23	356	30.9	20	339	25.0	21

From York (1994)
N, number of animals; SD, standard deviation; * $P < 0.005$; ** $P < 0.001$

Table 43. Females: Summary of body weight changes, F_0 lactation[a]

Days of lactation	Body weight changes (g)											
	0 ppm (control)			100 ppm			280 ppm			800 ppm		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
0–7	19	12.0	23	14	19.1	23	16	14.7	20	14	15.8	21
7–14	27	15.4	24	23	14.2	23	22	17.3	20	15	16.7	21
14–21	−31	17.5	24	−36	23.8	23	−21	16.6	20	−16	21.5	21
0–21	14	19.9	23	1	27.7	23	18	20.1	20	14	21.1	21

From York (1994)
N, number of animals; SD, standard deviation
[a] No statistical significance observed.

Table 44. Males: Summary of body weight values, F_1 weekly

Week of age	Body weight (g)											
	0 ppm (control)			100 ppm			280 ppm			800 ppm		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
4	103	6.9	26	105	11.8	26	103	11.2	26	91**	10.7	26
5	164	10.5	26	167	16.2	26	164	14.4	26	141**	16.5	26
6	223	12.6	26	227	17.6	26	225	19.6	26	194**	20.4	26
7	279	15.4	26	287	20.3	26	283	23.5	26	245**	23.5	26
8	336	19.3	26	350	25.9	26	343	30.7	26	298**	27.3	26
9	381	22.4	26	396	30.8	26	390	35.4	26	344**	32.4	26
10	417	26.0	26	435	35.6	26	425	38.9	26	374**	36.7	26
11	454	29.5	26	466	36.7	26	460	43.3	26	406**	40.4	26
12	469	28.1	26	491	37.9	26	481	46.6	26	428**	44.8	26
13	495	34.9	26	515	41.6	26	505	46.4	26	450**	46.9	26
14	515	42.4	26	540	48.5	26	526	46.9	26	467**	51.8	26
15	525	43.4	26	550	48.2	26	539	50.2	26	480**	51.7	26
16	537	41.8	26	564	52.2	26	554	49.8	26	493**	56.2	26
17	548	45.4	26	574	54.9	26	558	43.8	26	496**	57.9	26
18	553	49.5	26	580	58.9	26	566	44.5	26	507**	59.9	26
19	562	57.6	26	601*	63.7	26	583	48.0	26	522*	61.7	26
20	574	54.9	26	609	67.0	26	591	48.0	26	529*	66.2	26

Table 44 (continued)

Week of age	Body weight (g)											
	0 ppm (control)			100 ppm			280 ppm			800 ppm		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
21	586	58.5	26	624	66.9	26	604	54.5	26	539*	66.6	26
22	596	60.9	26	633	68.1	26	609	52.9	26	551*	66.2	26
23	601	61.0	25	649*	75.7	25	616	49.2	26	557*	72.0	26
24	614	62.0	24	663*	78.6	23	631	53.3	25	557*	72.3	23

From York (1994)
N, number of animals; SD, standard deviation; * $P < 0.005$; ** $P < 0.001$

Table 45. Females: Summary of body weight values, F_1 weekly

Week of age	Body weight (g)											
	0 ppm (control)			100 ppm			280 ppm			800 ppm		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	N
4	99	7.2	25	96	9.2	26	96	7.4	26	84**	7.8	25
5	145	10.6	25	141	12.1	26	143	8.6	26	127**	10.4	25
6	177	12.9	25	170	19.7	26	176	12.6	26	160**	10.8	25
7	198	16.0	25	191	24.4	26	198	15.1	26	183**	10.9	25
8	222	20.2	25	219	22.7	26	222	19.0	26	204**	13.4	25
9	240	22.6	25	233	27.4	26	240	21.0	26	220**	14.1	25
10	256	24.4	25	250	26.3	26	253	22.5	26	233**	14.6	25
11	267	26.8	25	264	32.5	26	268	25.3	26	245**	16.3	25
12	276	27.3	25	271	31.4	26	278	30.3	26	256*	16.8	25
13	285	28.9	24	282	32.8	25	285	30.4	26	264*	19.8	25
14	293	30.3	21	290	35.0	22	293	28.7	24	268*	16.8	21
15	308	36.7	6	309	27.7	10	306	24.3	10	279	17.0	3
16	333	27.4	3	329	28.5	8	337	24.6	6	307	43.1	2
17	341	43.1	3	330	42.8	5	357	40.4	5	396	NA	1
18	369	49.7	3	342	64.6	3	314	23.9	3	NA	NA	0
19	341	39.6	2	311	28.3	2	324	17.1	3	NA	NA	0
20	345	45.3	2	318	35.4	2	325	28.1	3	NA	NA	0

From York (1994)
N, number of animals; NA, not applicable; SD, standard deviation; * $P < 0.005$; ** $P < 0.001$

Table 46. Females: Summary of body weight values, F_1 gestation

Day of study	Body weight (g)											
	0 ppm (control)			100 ppm			280 ppm			800 ppm		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
0	292	26.9	18	286	34.4	19	296	26.8	16	267*	20.1	20
6	326	26.5	18	314	33.8	19	317	22.4	16	290**	18.8	20
15	372	28.0	18	356	31.3	19	357	23.0	16	326**	22.2	20
20	444	30.6	18	423	34.5	19	434	29.9	16	385**	28.9	20

From York (1994)
N, number of animals; SD, standard deviation; * $P < 0.005$; ** $P < 0.001$

Table 47. Females: Summary of body weight changes, F_1 gestation

Days of study	Body weight change (g)											
	0 ppm (control)			100 ppm			280 ppm			800 ppm		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
0–6	34	10.9	18	28	8.5	19	21**	11.5	16	23**	8.4	20
6–15	46	9.2	18	42	8.6	19	40	8.2	16	36**	9.6	20
15–20	72	11.4	18	68	17.5	19	77	14.3	16	59*	18.6	20
0–20	152	16.8	18	137	27.5	19	138	19.9	16	117**	21.1	20

From York (1994)
N, number of animals; SD, standard deviation; * $P < 0.005$; ** $P < 0.001$

Table 48. Females: Summary of body weight values, F_1 lactation

Day of lactation	Body weight (g)											
	0 ppm (control)			100 ppm			280 ppm			800 ppm		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
0	343	27.9	19	330	28.1	22	329	20.3	18	294**	21.5	21
7	357	27.6	19	349	23.9	22	345	24.0	18	315**	21.6	21
14	376	21.9	19	363	26.8	22	361	22.5	18	327**	21.9	21
21	351	22.2	19	347	24.0	22	343	17.7	18	335	20.1	21

From York (1994)
N, number of animals; SD, standard deviation; ** $P < 0.001$

Table 49. Females: Summary of body weight changes, F_1 lactation

Days of lactation	Body weight change (g)											
	0 ppm (control)			100 ppm			280 ppm			800 ppm		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
0–7	13	14.1	19	19	15.0	22	16	20.1	18	22	18.1	21
7–14	19	14.5	19	14	18.5	22	16	15.3	18	11	9.6	21
14–21	−24	12.4	19	−16	19.1	22	−18	17.6	18	8**	24.5	21
0–21	8	19.0	19	17	14.7	22	14	18.0	18	41**	28.0	21

From York (1994)
N, number of animals; SD, standard deviation; ** $P < 0.001$

Table 50. Summary of F_2 offspring growth

Dietary concentration (ppm)		Group mean body weight of live offspring during lactation (g)											
		Day of age											
		0		4BR		4AR		7		14		21	
		M	F	M	F	M	F	M	F	M	F	M	F
0	Mean	6.7	6.1	10.8	10.0	10.7	10.0	18.1	16.7	37.5	35.2	59.6	55.1
	SD	0.61	0.50	1.62	1.41	1.63	1.43	2.32	2.01	3.22	3.54	5.94	5.21
100	Mean	6.6	6.2	10.8	10.2	10.8	10.2	18.2	17.0	37.2	35.4	58.6	55.3
	SD	0.72	0.73	1.63	1.70	1.59	1.70	1.89	2.14	3.17	3.52	5.36	5.37

Table 50 (continued)

Dietary concentration (ppm)		Group mean body weight of live offspring during lactation (g)											
		Day of age											
		0		4BR		4AR		7		14		21	
		M	F	M	F	M	F	M	F	M	F	M	F
280	Mean	6.3	6.0	10.4	9.9	10.4	9.9	17.1	16.4	35.6	34.0	54.7	51.6
	SD	0.64	0.62	1.44	1.42	1.41	1.52	1.98	2.11	2.80	2.92	4.56	4.20
800	Mean	6.1	5.8	9.4	8.9	9.4	8.9	15.6*	14.6	31.6*	29.8*	48.7*	45.5*
	SD	0.74	0.80	1.75	1.55	1.77	1.53	2.69	2.64	3.61	4.55	4.60	5.94

AR, after reduction; BR, before reduction; F, females; M, males; SD, standard deviation
* Significantly different from control group, $P \leq 0.05$

Table 51. Females: Summary of feed consumption values, F_0 gestation

Days of study	Feed consumption (g/animal per day)											
	0 ppm (control)			100 ppm			280 ppm			800 ppm		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
0–6	18.8	1.87	25	18.8	1.87	25	17.5*	1.98	25	15.8**	1.39	25
6–15	31.0	3.86	25	30.8	3.28	25	28.2*	3.80	25	27.5**	3.35	25
15–20	18.2	3.20	25	18.1	2.51	25	17.7	2.66	24	16.5	2.42	25
0–20	22.6	2.7	50	22.5	2.03	50	21.2**	2.40	50	19.9**	2.04	51

From York (1994)
N, number of animals; SD, standard deviation; * $P < 0.005$; ** $P < 0.001$

Table 52. Females: Summary of feed consumption values, F_0 lactation

Days of lactation	Feed consumption (g/animal per day)											
	0 ppm (control)			100 ppm			280 ppm			800 ppm		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
0–7	41.8	3.77	24	39.2	4.02	23	40.4	3.24	19	38.2*	4.9	21
7–14	67.9	17.19	23	61.1	7.52	23	61.9	3.89	19	56.8*	6.57	21
14–21	77.4	5.35	24	74.7	5.29	23	78.3	11.62	20	72.2*	4.48	20
0–21	62.2	7.4	24	58.4	4.65	23	60.7	5.83	20	55.4**	5.47	21

From York (1994)
N, number of animals; SD, standard deviation; * $P < 0.005$; ** $P < 0.001$

Table 53. Females: Summary of feed consumption values, F_1 gestation

Days of gestation	Feed consumption (g/animal per day)											
	0 ppm (control)			100 ppm			280 ppm			800 ppm		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
0–6	23.7	2.67	18	21.6*	2.92	19	20.7**	2.38	16	19.5**	2.54	20
6–15	26.8	2.20	18	24.5**	2.32	19	23.7**	1.77	16	22.4**	2.73	20
15–20	26.9	2.57	18	26.0	2.64	19	25.7	3.19	16	23.8**	3.29	20
0–20	25.9	2.1	18	24*	2.14	19	23.3**	1.85	16	21.9**	2.15	20

From York (1994)
N, number of animals; SD, standard deviation; * $P < 0.005$; ** $P < 0.001$

Table 54. Females: Summary of feed consumption values, F_1 lactation

Days of lactation	Feed consumption (g/animal per day)											
	0 ppm (control)			100 ppm			280 ppm			800 ppm		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
0–7	39.0	5.24	19	38.9	5.53	22	36.9	5.09	17	36.5	6.94	21
7–14	61.5	6.43	18	58.0	12.29	21	56.2*	3.95	18	51.6**	7.6	20
14–21	72.0	7.80	19	70.4	13.39	22	68.4	5.67	15	62.6*	11.66	20
0–21	57.5	5.9	19	55.9	10.02	22	53.1	6.46	18	49.8**	8.29	21

From York (1994)
N, number of animals; SD, standard deviation; * $P < 0.005$; ** $P < 0.001$

weaning index, which measures offspring survival between lactation day 4 after culling and lactation day 21, was also reduced in this treated group when compared with that of the control group.

The treatment-related microscopic changes in the liver noted in the high-dose group, hepatocyte hypertrophy (F_0 and F_1 generations) and vacuolar change in hepatocytes (F_1 generation), were also observed for the mid-dose males. The severity of these changes was less in the mid-dose group than in the high-dose group (Tables 55–58).

There were no adverse effects on offspring development in litters in the mid-dose group in either generation.

There were apparent reductions in the F_0 male and female fertility indices and in the pregnancy index in the mid- and high-dose groups in comparison with the concurrent control group, with the difference being statistically significant in the mid-dose group. However, there were no dose-related differences in the F_1 male and female fertility indices and in the pregnancy index between the control and these treated groups. In the F_0 generation, the control group had values of 100% for these indices, whereas in the F_1 generation, there were values of 76% and 82% for the fertility and pregnancy indices, respectively, in the control group. All of these values, and the values in the treated groups in both generations, were within the range of values in the historical control data. It is therefore questionable if the variations between the control and mid- and high-dose groups in the F_0 generation represent an adverse test article–related effect on reproductive performance.

For the F_0 males that failed to sire a litter in the treated groups, the sperm were generally at least 50% motile, and a minimum of 92% of the sperm examined from each male were morphologically normal. As spermatogenesis was not evaluated for any control males (all sired litters) or for any fertile males, assessment of sperm concentration values of the non-fertile treated males for adverse effects is difficult. However, only one male in the mid-dose group had a concentration sufficiently low that morphological assessment could not be made. The values are presented in Table 59.

No adverse effects on reproductive performance or offspring development were evident at the low dose level.

A supplementary reproductive assessment was done. As the epididymides of F_0 high-dose males were autolysed due to an error, a complete reproductive assessment was not obtained for that dose (800 ppm) in the two-generation study. In this supplementary study, only two groups of male rats were taken (group I, control; group II, 800 ppm) for study, and acetamiprid was administered through the diet at a dose of 800 ppm to group II animals for 20 weeks, a duration comparable to that for the F_0 males in the two-generation study. Group I animals were given food ad libitum.The males were monitored for clinical signs, body weight changes, feed consumption and general condition throughout the study. At termination, organ weights were obtained, and a reproductive capacity evaluation, which included total testicular and epididymal sperm counts and sperm morphology, was perfomed.

Table 55. Incidence of microscopic observations in liver, terminal sacrifice of F_0 rats (males)

Tissue observation	0 ppm (control)		100 ppm		280 ppm		800 ppm	
	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC
Number of animals examined	*0*	*26*	*0*	*26*	*0*	*26*	*0*	*26*
Number of animals within normal limits	*0*	*23*	*0*	*25*	*0*	*5*	*0*	*0*
Extramedullary haematopoiesis, trace	0	1	0	0	0	0	0	1
Inflammation, trace	0	0	0	1	0	0	0	1
Necrosis, mild	0	1	0	0	0	0	0	0
Vacuolar change	0	1	0	0	0	2	0	3
- trace	0	1	0	0	0	2	0	2
- mild	0	0	0	0	0	0	0	1
Hepatocyte hypertrophy	0	0	0	0	0	21**	0	26**
- trace	0	0	0	0	0	21	0	0
- mild	0	0	0	0	0	0	0	16
- moderate	0	0	0	0	0	0	0	10

From York (1994)
DOS, death on study; SAC, terminal sacrifice; ** significantly different from control (DOS and SAC combined): $P \leq 0.01$

Table 56. Incidence of microscopic observations in liver, terminal sacrifice of F_0 rats (females)

Tissue observation	0 ppm (control)		100 ppm		280 ppm		800 ppm	
	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC
Number of animals examined	*0*	*26*	*0*	*26*	*0*	*26*	*0*	*26*
Number of animals within normal limits	*0*	*25*	*0*	*25*	*0*	*25*	*0*	*4*
Extramedullary haematopoiesis, trace	0	0	0	0	0	1	0	1
Inflammation	0	1	0	0	0	0	0	2
- trace	0	1	0	0	0	0	0	1
- mild	0	0	0	0	0	0	0	1
Necrosis, trace	0	0	0	1	0	0	0	1
Hepatocyte hypertrophy	0	0	0	0	0	0	0	22**
- trace	0	0	0	0	0	0	0	6
- mild	0	0	0	0	0	0	0	16

From York (1994)
DOS, death on study; SAC, terminal sacrifice; ** significantly different from control (DOS and SAC combined): $P \leq 0.01$

Table 57. Incidence of microscopic observations in liver, day 0 to termination, F_1 rats (males)

Tissue observation	0 ppm (control)		100 ppm		280 ppm		800 ppm	
	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC
Number of animals examined	*0*	*26*	*0*	*26*	*0*	*26*	*0*	*26*
Number of animals within normal limits	*0*	*25*	*0*	*25*	*0*	*1*	*0*	*0*
Extramedullary haematopoiesis, trace	0	0	0	0	0	0	0	1
Inflammation, trace	0	1	0	0	0	0	0	0
Necrosis, mild	0	0	0	0	0	1	0	0
Vacuolar change	0	0	0	0	0	2	0	12**

Table 57 (continued)

Tissue observation	0 ppm (control)		100 ppm		280 ppm		800 ppm	
	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC
- trace	0	0	0	0	0	2	0	9
- mild	0	0	0	0	0	0	0	3
Hepatocyte hypertrophy	0	0	0	1	0	25**	0	26**
- trace	0	0	0	1	0	21	0	0
- mild	0	0	0	0	0	4	0	11
- moderate	0	0	0	0	0	0	0	15

From York (1994)
DOS, death on study; SAC, terminal sacrifice; ** significantly different from control (DOS and SAC combined): $P \leq 0.01$

Table 58. Incidence of microscopic observations in liver day 0 to termination, F_1 rats (females)

Tissue observation	0 ppm (control)		100 ppm		280 ppm		800 ppm	
	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC
Number of animals examined	*1*	*25*	*0*	*26*	*0*	*26*	*1*	*25*
Number of animals within normal limits	*1*	*21*	*0*	*26*	*0*	*26*	*1*	*5*
Extramedullary haematopoiesis, trace	0	4	0	0	0	0	0	3
Hepatocyte hypertrophy	0	0	0	0	0	0	0	20**
- trace	0	0	0	0	0	0	0	13
- mild	0	0	0	0	0	0	0	7

From York (1994)
DOS, death on study; SAC, terminal sacrifice; ** significantly different from control (DOS and SAC combined): $P \leq 0.01$

Table 59. Summary of F_0 male spermatogenesis data

	100 ppm			280 ppm			800 ppm		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
Sperm motility (%)	71	13.6	3	61	33.4	6	53	35.6	5
Sperm concentration (number/g caudal tissue)	761.1	442.94	3	797.2	492.29	6	716.7	523.17	5
% abnormal	2.0	1.5	3	1	0.7	5	3	3.1	5

From York (1994)
N, number of animals; SD, standard deviation

There were no deaths attributable to administration of the test material. One 800 ppm male was sacrificed in extremis during week 16. No compound-related effects were seen.

Mean body weight and feed consumption values were consistently lower in the 800 ppm group throughout the study. The lower body weights of the test group males relative to control values correlated to their lower feed consumption. Feed efficiency was similar between control and test groups. There were no compound-related effects in the evaluation of organ weights of the 800 ppm males. Also, no effects were observed in the evaluation of testicular and epididymal sperm count and sperm morphology in this group.

In conclusion, dietary exposure of male rats to acetamiprid at a dose level of 800 ppm for at least 20 weeks did not result in effects on reproductive capacity (Trutter, 1999).

Keeping in view the above, the NOAEL for systemic adult toxicity when acetamiprid was administered to Charles River Crl:CD VAF/Plus rats was 100 ppm (equal to 6.67 mg/kg bw per

day), on the basis of a decline in body weights and feed consumption and an increased incidence of hepatocyte hypertrophy and vacuolation in the liver at 280 ppm (equal to 18.9 mg/kg bw per day) and above. The NOAEL for offspring toxicity was 280 ppm (equal to 13.9 mg/kg bw per day), on the basis of decreases in body weight gain in both generations and reduced postnatal survival in the F_2 offspring at 800 ppm (equal to 38.7 mg/kg bw per day). The NOAEL for reproductive toxicity was 800 ppm (equal to 38.7 mg/kg bw per day), the highest dose tested.

This study was conducted in compliance with GLP, and a QA statement was attached (York, 1994, 1995).

(b) Developmental toxicity

Rats

A study was conducted to assess the potential maternal toxicity, fetal toxicity and teratogenicity of acetamiprid in pregnant rats. Acetamiprid (lot No. NNI-02, purity 99.46%) suspensions in vehicle (5% gum arabic and 0.01% Tween 80 in water) were administered orally by gastric intubation to Cij:CD(SD) rats (24 mated females per group) at dose levels of 5, 16 and 50 mg/kg bw per day for a period of 10 days from gestation days 6 to 15. Control animals received vehicle (0.2 ml/100 g bw per day) only, in the same procedure as treated animals.

The mated females were observed for clinical signs once a day from the first mating to necropsy. Individual body weights (on gestation days 0 and 6–21) and feed consumption (on days 6–21) were measured once a day.

On gestation day 21, all mated females were sacrificed, and their organ weights (liver, spleen, kidney, adrenal and ovary) were measured. The number of corpora lutea was counted. After the gravid uterus was weighed, the numbers of total implantations, viable fetuses, dead fetuses and resorbed fetuses were counted. Viable fetuses were examined for external malformations and variations, and their body weight and placental weight were recorded. One half of the viable fetuses in each litter were used for visceral evaluation using the method of Wilson and Nishimura, and the remaining fetuses were used for skeletal evaluation (McLeod method).

Acetamiprid did not cause toxic signs or deaths in any groups. Pregnancy rates for the control, 5, 16 and 50 mg/kg bw per day groups were 95.8%, 95.8%, 100% and 95.8%, respectively.

Effects of acetamiprid treatment on body weight, feed consumption and liver weights were seen in the high-dose (50 mg/kg bw per day) group. Mean body weights for the high-dose animals were lower than control values throughout the treatment period; differences were statistically significant ($P < 0.05$ or $P < 0.01$) on gestation days 8–16. Their cumulative body weight gains during the treatment period were significantly lower ($P < 0.01$, reduced by 41%) than control values. Feed consumption of the high-dose group was lower (reduced by 2.45%) than control values throughout the study; differences were statistically significant ($P < 0.01$) on gestation days 7–11. Increases in liver weights ($P < 0.05$) and liver to body weight ratios ($P < 0.01$) of the high-dose group were statistically significant.

No effects of acetamiprid treatment on the numbers of total implantation sites, corpora lutea and viable fetuses were evident.

Macroscopically, no adverse effect of acetamiprid treatment was evident. The following two lesions were seen in one animal each, but they were not considered to be related to treatment: splenodiaphragmatic adhesion in a 16 mg/kg bw per day animal and splenic hypertrophy in a 50 mg/kg bw per day animal.

Observations of the maternal reproductive system revealed no adverse effect of acetamiprid treatment. No immature delivery or abortion was seen in any treated group, including the 50 mg/kg bw per day group. The number of resorbed fetuses (early death) and post-implantation loss values for the 16 mg/kg bw per day group were significantly increased, relative to control values, but were

not considered to be related to acetamiprid treatment, because there was no treatment relationship ($P = 0.12$ and $P = 0.11$, not significant). There was no adverse effect of acetamiprid treatment on the uterine weight.

No significant change, relative to control values, was noted in the numbers of male and female fetuses per pregnant female, sex ratio or fetal placental weight.

The only external malformation noted was short tail in one fetus from the litter of one control female (incidence 0.3%). The external variations seen were subcuticular and placental haemorrhage. Based on litter analysis, the incidences of subcuticular haemorrhage for the control, 5, 16 and 50 mg/kg bw per day groups were 8.7% (2/23 litters), 4.3% (1/23 litters), 4.2% (1/24 litters) and 17.4% (4/24 litters). The placental haemorrhage was seen in three fetuses from two litters of the 50 mg/kg bw per day group (0.9% for the fetal incidence and 8.7% for the maternal incidence). The incidences in each group were not statistically significantly different either for the placental haemorrhage or for the fetal subcuticular haemorrhage. After reanalysis of values of the low and intermediate doses only for a trend effect, there was no dose-dependent incidence of these parameters. Acetamiprid treatment was not considered to cause external modifications of fetuses.

Acetamiprid treatment did not increase the incidences of fetuses with visceral malformations and/or variations.

There were no visceral malformations in the control and treated groups.

The incidences of fetuses with visceral variations for the control, 5, 16 and 50 mg/kg bw per day groups were 12.6% (22/174 fetuses), 8.6% (15/175 fetuses), 13.3% (24/180 fetuses) and 8.0% (13/162 fetuses), respectively; the incidences, relative to control values, were not statistically significant. The main visceral variations seen in fetuses of the control and treated groups were dilatation of the renal pelvis and thymic remnants in the neck. The incidences of fetuses with dilatation of the renal pelvis for the control, 5, 16 and 50 mg/kg bw per day groups were 7.5% (13/174 fetuses), 4.6% (8/175 fetuses), 10.6% (19/180 fetuses) and 6.2% (10/162 fetuses), respectively. The incidences of fetuses with thymic remnants in the neck were 4.6% (8/174 fetuses), 4.0% (7/175 fetuses), 2.8% (5/180 fetuses) and 1.9% (3/162 fetuses), respectively. No statistical significance was attained in the treated groups, compared with control values. The low incidences of other visceral variations seen in the treated groups were not considered to be indicative of treatment-related responses. These results suggest that acetamiprid did not affect visceral variations.

Acetamiprid treatment did not cause skeletal malformations, variations or retarded ossification in fetuses. The incidences of fetuses with skeletal malformations for the control, 5, 16 and 50 mg/kg bw per day groups were 1.8% (3/171 fetuses), 0% (0/165 fetuses), 1.1% (2/175 fetuses) and 0.6% (1/162 fetuses), respectively. The incidences of litters containing fetuses with skeletal malformations for these same groups were 13% (3/23 litters), 0% (0/23 litters), 8.3% (2/24 litters) and 4.5% (1/22 litters), respectively. There were no statistically significant differences in the incidences of skeletal malformations, on either a per fetus or per litter basis, when compared with control values. The main skeletal malformations seen in fetuses of the control and treated groups were defect of post-cervical vertebrae, partial hypertrophy, fusion and bifurcation of the ribs and fusion of the sternums. Partial hypertrophy of the rib was seen in one fetus from each of the control, 16 and 50 mg/kg bw per day groups. In the control group, three fetuses had defect of post-cervical vertebrae, fusion or bifurcation of the ribs. Fusion of the sternums was seen in one fetus from the 16 mg/kg bw per day group. These incidences of fetuses with skeletal malformations were considered comparable to control values. These results suggest that there was no effect of acetamiprid treatment on skeletal malformation.

The incidences of fetuses with skeletal variations for the control, 5, 16 and 50 mg/kg bw per day groups were 21.6% (37/171 fetuses), 28.5% (47/165 fetuses), 25.1% (44/175 fetuses) and 29.0% (47/162 fetuses), respectively. The main skeletal variations seen in fetuses of the control and treated groups were bibbed shape of the thoracic vertebral body, splitting of the cervical vertebral body,

shortening of the 13th rib, 14th rudimentary rib and asymmetry of the sternum. The incidences of fetuses with bibbed shape of the thoracic vertebral body, splitting of the cervical vertebral body, 14th rudimentary rib and asymmetry of the sternum in the treated groups were comparable to or less than control values. The incidence of fetuses with 14th rudimentary rib from the 50 mg/kg bw per day group (0/162 fetuses) was lower than the control value (8/171 fetuses) and was inversely dose dependent ($P < 0.01$). The incidences of fetuses (and litters) with shortening of the 13th rib for the control, 5, 16 and 50 mg/kg bw per day groups were 0.6% (1/171 fetuses, 1/23 litters), 3.6% (6/165 fetuses, 3/23 litters), 0.6% (1/175 fetuses, 1/24 litters) and 9.3% (15/162 fetuses, 8/22 litters), respectively. Statistical significance was observed at 50 mg/kg bw per day on both a fetus ($P < 0.01$) and litter ($P < 0.01$) basis for the shortening of the 13th rib compared with the control group.

There was no effect of acetamiprid treatment on the ossification process.

No effects of acetamiprid treatment were evident in the numbers of total implantations.

The NOAEL for maternal toxicity in rats was 16 mg/kg bw per day, based on decreases in feed consumption and body weight gain during the treatment period noted in maternal rats in the 50 mg/kg bw per day group at scheduled sacrifice. The developmental NOAEL in rats was 16 mg/kg bw per day, based on the increased incidences of fetuses with shortening of the 13th rib at 50 mg/kg bw per day.

This study was conducted in compliance with GLP, and a QA statement was attached (Nukui & Fujii, 1994a).

Rabbits

In a developmental toxicity study, acetamiprid (lot No. NNI-02, purity 99.46%) suspended in vehicle (5% gum arabic and 0.01% Tween 80 in water) was administered orally by gastric intubation to Kbs: NZW rabbits (17 mated females per group) at dose levels of 7.5, 15 and 30 mg/kg bw per day for a period of 13 days from gestation days 6 to 18. Control animals received vehicle (4 mg/kg bw per day) only in the same procedure as treated animals.

The age of the animals was 5 months at the time of receipt, and the mean weight of males was 4111.4 ± 248.9 g (range 3547.9–4513.7 g) and of females was 3659.8 ± 265.0 g (range 3193.8–4298.3 g).

The mated females were observed for clinical signs once a day from the first mating to necropsy. Individual body weights (on gestation days 0, 6, 7, 8, 10, 12, 14, 16, 18, 19, 20, 22, 24 and 28) and daily feed consumption (on days 6, 7, 8, 10, 12, 14, 16, 18, 19, 20, 22, 24 and 28) were measured.

On gestation day 28, all mated females were sacrificed, and their organ weights (liver, spleen, kidney, adrenal and ovary) were measured. The number of corpora lutea was counted. After the gravid uterus was weighed, the numbers of total implantations and viable, dead and resorbed fetuses were counted. Viable fetuses were examined for external malformations and variations, and their body weights and placental weights were recorded. After fixation with ethanol, each fetus was used for visceral and skeletal evaluation.

Acetamiprid did not cause death in any treated animal. As a result of inadequate treatment or restraint, three, two and one animal from the 7.5, 15 and 30 mg/kg bw per day groups, respectively, died. The mortalities for the control, 7.5, 15 and 30 mg/kg bw per day groups were 0% (0/17 dams), 17.6% (3/17 dams), 11.8% (2/17 dams) and 5.9% (1/17 dams), respectively. Pregnancy rates for the control, 7.5, 15 and 30 mg/kg bw per day groups were 70.6%, 88.2%, 82.4% and 82.4%, respectively.

One control animal and one treated animal of the 7.5 mg/kg bw per day group showed prone position and abnormal gait (lumber paralysis), respectively. These signs were not considered to be related to treatment, because the former animal had lung lesions, and the latter died from inadequate restraint.

Treatment-related effects on maternal body weight were evident. Following the first treatment, maternal growth in the control, 15 and 30 mg/kg bw per day groups was depressed. Maternal body

weights for the control and 15 mg/kg bw per day groups were temporarily decreased from the first treatment to day 10 or 12 and recovered to the pretreatment level by gestation day 14 or 16. In the 30 mg/kg bw per day group, maternal body weights on day 19 were still lower than the pretreatment level, and body weight gain during the treatment period (gestation days 6–19) was −7.3 g (35.2 g in the control group).

During the post-treatment period (gestation days 19–28), body weight gains for the 7.5 and 15 mg/kg bw per day groups were lower than those for the control group. However, they were not considered to be related to the treatment, because no statistical significance was noted in these same groups, and body weight gains for the 30 mg/kg bw per day group were comparable to those for the control group.

Treatment-related effects on maternal feed consumption were evident. Oral administration of acetamiprid at a dose level of 30 mg/kg bw per day resulted in a tendency to reduced mean feed consumption during the treatment period. Statistically significant reductions in feed consumption, relative to control values, were noted for the 30 mg/kg bw per day group on gestation days 6–7 and 7–8. In the 30 mg/kg bw per day group, decreased body weights were noted simultaneously, and the decreased feed consumption was considered to be related to acetamiprid treatment. During the post-treatment period, mean feed consumption for the 30 mg/kg bw per day group was comparable to that for the control group. In other groups, feed consumption during the pretreatment, treatment and post-treatment period was comparable to the control group values.

No effect of acetamiprid on maternal organ weights was evident.

Macroscopically, no treatment-related effect was evident. In animals that died accidentally, the following lesions were seen: perforation, dark red areas, red patches in the lung and red fluid on the tracheal mucosa and in the thoracic cavity. These lesions were attributable to improper treatment. In the dams that survived until gestation day 28, pale-brown areas and black patches in the lung, red fluid on the tracheal mucosa and prominent hepatic lobules were seen occasionally in each group, including the control group.

No adverse effect of acetamiprid treatment on the maternal reproductive system was evident. No premature delivery or abortion was seen in any treated group, including the 30 mg/kg bw per day group. No adverse effect on the numbers of total implantations, corpora lutea, or viable and non-viable fetuses or on the values of pre-implantation and post-implantation losses was noted.

No significant changes, relative to control values, were noted in fetal body weights, placental weights and numbers of male and female fetuses per pregnant female for the treated groups. The incidences of underdeveloped fetuses in the treated groups were higher than the control values, but there was no statistical significance.

External malformations, open eyelid and manus varus, were seen in one fetus from the litter of one 30 mg/kg bw per day female (incidence 1.2%, 1/86 fetuses). External variations were not seen in any group. The external malformations seen in the 30 mg/kg bw per day group were not considered to be related to treatment because the incidence was not statistically significant.

No effects of treatment on visceral malformation or variation were seen in fetuses. The incidences of fetuses with visceral malformations for the control, 7.5, 15 and 30 mg/kg bw per day groups were 1.1% (1/93 fetuses), 1.0% (1/99 fetuses), 0% (0/94 fetuses) and 1.2% (1/86 fetuses), respectively. The incidences of dams containing fetuses with visceral malformations for these same groups were 8.3% (1/12 dams), 8.3% (1/12 dams), 0% (0/12 dams) and 7.7% (1/13 dams), respectively. There were no statistically significant differences in the incidences of visceral malformations, on either a per fetus or per dam basis, when compared with control data. The visceral malformations seen in fetuses of the control and treated groups were microphthalmia (30 mg/kg bw per day), abnormal origin of right subclavian artery (control) and bifid apex of heart (7.5 mg/kg bw per day).

The incidences of fetuses with visceral variations for the control, 7.5, 15 and 30 mg/kg bw per day groups were 3.2% (3/93 fetuses), 3.0% (3/99 fetuses), 5.3% (5/94 fetuses) and 2.3% (2/86 fetuses), respectively. These incidences were not statistically significant compared with control values. The visceral variations seen in fetuses from the control and treated groups were dilatation of renal pelvis and thymic remnant in the neck.

Acetamiprid did not cause fetal skeletal malformations, variations or ossification defects. The incidences of fetuses with skeletal malformations for the control, 7.5, 15 and 30 mg/kg bw per day groups were 4.3% (4/93 fetuses), 4.0% (4/99 fetuses), 3.2% (3/94 fetuses) and 4.7% (4/86 fetuses), respectively. The incidences of dams containing fetuses with skeletal malformations for these same groups were 33.3% (4/12 dams), 25.0% (3/12 dams), 25.0% (3/12 dams) and 30.8% (4/13 dams), respectively. There were no statistically significant differences in the incidences of skeletal malformations, on either a per fetus or per dam basis, when compared with control data. The main skeletal malformations seen in fetuses of the control and treated groups were absence and fusion of thoracic vertebral arches, absence of thoracic vertebral body, fusion of ribs, bifurcation of rib, fusion of sternebrae and absence of lumbar vertebral arch. The fusion of thoracic vertebral arches was seen only in the 30 mg/kg bw per day group (2/86 fetuses), with a dose–response relationship ($P < 0.05$). However, this effect was not considered to be related to the treatment, because the incidence was not statistically significant when compared with the control values.

The incidences of fetuses with skeletal variations for the control, 7.5, 15 and 30 mg/kg bw per day groups were 71.0% (66/93 fetuses), 64.6% (64/99 fetuses), 45.7% (43/94 fetuses) and 69.8% (60/86 fetuses), respectively. The skeletal variations seen in fetuses from the control and treated groups were cervical rib, asymmetry, splitting and bilobed shape of the sternebrae, shortened 13th rib, nodulated rib, 13th rib and floating rib. The most frequent variation was the 13th rib. Morita et al. (1987) reported that the incidences of the 13th rib in New Zealand White rabbits were higher than those of other rabbit strains. The 13th ribs seen in this study were also considered to be spontaneous. The incidence of the 13th rib in the 15 mg/kg bw per day group was significantly lower than the control incidence. However, this is not considered to be related to acetamiprid treatment, because no statistical significance was noted in the higher dose group. The incidence of floating rib in the 30 mg/kg bw per day group (4/86 fetuses) was significantly lower than the control incidence (14/93 fetuses), with a negative dose–response relationship ($P < 0.05$). The incidence of shortened 13th rib in the 7.5 mg/kg bw per day group (16.2%, 16/99 fetuses) was significantly higher than the control incidence (3.2%, 3/93 fetuses). However, this was not considered to be related to acetamiprid treatment, because there was no dose–response relationship ($P = 0.68$, not significant). The incidences of other variations lacked statistical significance compared with the control group. Based on the findings mentioned above, it was considered that acetamiprid treatment did not cause fetal skeletal variations. Acetamiprid treatment did not cause retarded ossification in fetuses of any treated group.

The NOAEL for maternal toxicity in rabbits was 15 mg/kg bw per day, based on the decreases in feed consumption and body weight gain during the treatment period noted at 30 mg/kg bw per day. The developmental NOAEL was 30 mg/kg bw per day, the highest dose tested.

This study was conducted in compliance with GLP, and a QA statement was attached (Nukui & Fujii, 1994b).

2.6 Special studies

(a) Neurotoxicity

Acute neurotoxicity

A dose range–finding study was undertaken for the assessment of behavioural changes and time for peak effect for these changes following treatment with acetamiprid. Three groups of three

male and three female Crl:CD(SD)BR rats were dosed with acetamiprid (batch No. NFG-02, purity 99.9%) on a single occasion at a dose of 10, 50 or 100 mg/kg bw. The animals were 35 days old with weights of approximately 15 g at the time of receipt and acclimatized for 6 days before initiation of the study. A functional observational battery was performed prior to treatment and 0.5, 2 and 5 hours after dosing. Body weight was recorded on a daily basis after treatment. Animals were maintained for 14 days after treatment and then killed and subjected to routine macroscopic examination.

At 100 mg/kg bw, there were clinical signs of dilated pupil (females only) and tremors and an initial impairment in body weight gain for females on day 1. Neurobehavioural observations included body tremors, chewing (females only), hunched posture and clear reduction in body temperature. At 50 mg/kg bw, neurobehavioural observations included body tremors and tail tremors (females only). There were no clear effects of treatment at 10 mg/kg bw. The time for peak effect was between 5 and 6 hours post-dosing.

In view of the above, a dose of 100 mg/kg bw was suggested to be a suitable highest dose for the subsequent acute neurotoxicity study, with post-dosing observations being performed at approximately 6 hours after dosing.

This study was not conducted in compliance with GLP (Hughes, 1997a).

Assessment of the potential neurotoxicity of acetamiprid in male and female rats was studied. Three groups of 10 male and 10 female Crl:CD BR rats (approximately 35 days old for males and 28 days old for females and weighing approximately 15 g for each sex) were given acetamiprid (batch No. NFG-02, purity 99.9%) as a single oral (by gavage in 0.5% sodium carboxymethylcellulose) dose at levels of 10, 30 and 100 mg/kg bw. A similar size group was given the vehicle and acted as control.

Throughout the study, clinical signs, body weight and feed consumption were monitored. All animals were subjected to a functional observational battery prior to treatment, at 6 hours post-dosing and on days 7 and 14 post-dosing. The motor activity of each animal was also quantitatively assessed prior to treatment, at 6 hours post-dosing and on days 7 and 14 post-dosing. At the end of the treatment period, all animals were killed using whole-body perfusion. Examination was confined to designated tissues of the nervous system, which were subsequently examined microscopically.

With respect to the clinical signs, signs of reaction to treatment were confined to the day of dosing at 100 mg/kg bw and were observed 7–8 hours after dosing only. Signs included tremor, hunched posture, unsteadiness and coldness to touch. These were the same signs that were noted during the performance of the functional observational battery (Table 60).

Feed consumption among males of the 100 mg/kg bw group was significantly lower than that of controls in the 1st week following administration. The gains in week 2 were comparable to those of controls. No such findings could be seen among females of the highest-dose group (Table 61).

Body weight gains for males at 100 mg/kg bw were statistically significantly lower compared with controls in the 1st week following the acute administration. The gains in week 2 were comparable with those for controls. There were no similar findings among females at 100 mg/kg bw. At lower doses, there was no effect on weight gain (Table 62).

There was no difference in feed conversion ratios between treated and control groups.

Functional observational data revealed no remarkable differences between the groups either in terms of the observational end-points or in terms of the continuous measurements prior to dosing.

Six hours post-dosing, a range of effects was observed. Tremors were observed among males and females treated at 100 mg/kg bw. A few animals were observed with tremors in the cage (females only) or on removal from the cage (males and females). Although these observations of tremor were not always supported by statistically significant differences, the occurrences of tremors in the cage and in the hand has never been seen in control animals, and it was considered to be attributable to treatment. Most of the tremors were observed once the animals were placed into the arena. Tremors

Table 60. Principal clinical signs associated with treatment

Signs observed	Number of animals in groups showing signs							
	Males				Females			
	Dose (mg/kg bw)							
	Control	10	30	100	Control	10	30	100
Tremor	0	0	0	3	0	0	0	4
Unsteady	0	0	0	2	0	0	0	1
Reluctant to move	0	0	0	0	0	0	0	1
Hunched	0	0	0	1	0	0	0	3
Cold to touch	0	0	0	0	0	0	0	2
Nasal staining	0	0	0	0	0	0	0	1

From Hughes (1997b)

Table 61. Feed consumption, group mean values

Week	Feed consumption, mean ± standard deviation (g)			
	Dose (mg/kg bw)			
	Control	10	30	100
Males				
−1	205	207	205	199
1	233 ± 12.7	237 ± 17.9	234 ± 22.1	211* ± 24.6
2	231 ± 8.4	231 ± 17.1	235 ± 20.5	223 ± 21.5
Females				
−1	139	137	143	141
1	173 ± 10.8	180 ± 12.7	182 ± 9.2	172 ± 6.9
2	175 ± 18.3	176 ± 11.0	175 ± 12.9	179 ± 11.5

From Hughes (1997b)
* $P < 0.05$

ranged from moderate to marked and affected the body, head, limbs and tail. They appeared to be exacerbated by the animal moving, and on some occasions, for some females, the animals appeared to collapse for a brief period following a bout of tremor. The incidence of tremors in the arena was statistically significantly increased among females at 100 mg/kg bw. Although not statistically significant, the occurrence of marked tremors in males at 100 mg/kg bw was attributed to treatment (Table 63).

Chewing movements of the mouth were observed in one male at 100 mg/kg bw, one female at 10 mg/kg bw and five females at 100 mg/kg bw. The latter incidence was statistically significant. Males at 100 mg/kg bw were statistically significantly more difficult to handle on removal from their cage. Some males and females at 100 mg/kg bw were cold to the touch, with the incidence among females attaining statistical significance.

Pupils were observed as being dilated just prior to the pupil reflex in three males and six females at 100 mg/kg bw. These same animals were assessed as having medium to large pupil size under normal light. These incidences were statistically significant.

There was an indication that frequency of urination was increased among males treated at 30 and 100 mg/kg bw, with the incidence among males at 100 mg/kg bw attaining statistical significance.

Table 62. Body weight, group mean values

Weeks	Dose (mg/kg bw)			
	Control	10	30	100
Males				
Body weight (g)				
−1	172	173	172	172
0	201	205	201	199
1	278	283	281	265
2	322	322	322	305
Body weight gain, mean ± standard deviation (g/rat per week)				
0–1	77 ± 6.3	78 ± 5.4	80 ± 8.5	66* ± 14.4
1–2	43 ± 5.5	39 ± 7.5	41 ± 8.6	41 ± 7.9
0–2	121 ± 10.7	117 ± 12.1	121 ± 15.6	106* ± 20.2
% of control				
0–1	—	101	104	86
1–2	—	91	95	95
0–2	—	97	100	88
Females				
Body weight (g)				
−1	153	154	155	155
0	154	155	157	158
1	193	199	200	200
2	215	221	220	224
Body weight gain, mean ± standard deviation (g/rat per week)				
0−1	39 ± 6.3	45 ± 5.0	43 ± 5.8	42 ± 6.8
1−2	22 ± 5.4	21 ± 6.2	20 ± 7.9	24 ± 3.9
0−2	61 ± 7.0	66 ± 5.6	63 ± 7.7	66 ± 7.6
% of control				
0−1	—	115	110	108
1−2	—	95	91	109
0−2	—	108	103	108

From Hughes (1997b)
* $P < 0.05$

There was hunched posture in males treated at 100 mg/kg bw. A low incidence of hunched posture was observed in all treated females, with the highest incidence occurring among females treated at 100 mg/kg bw. This incidence was statistically significantly increased compared with controls. An increased incidence of walking on toes was noted in males treated at 100 mg/kg bw and in all treated female groups. The incidence among males was statistically significantly increased.

Slipping of hindlimbs was observed in one male and three females at 100 mg/kg bw. The incidence among females was statistically significantly increased.

Two females at 100 mg/kg bw were unable to lift their front feet and were recorded as “front feet dragging”. A female was assessed as having impaired mobility due to the changes in gait.

Table 63. Functional observational data (tremor)

	Incidence of tremor															
	Pre-dosing								6 h post-dosing							
	Males				Females				Males				Females			
	Dose (mg/kg bw)															
	0	10	30	100	0	10	30	100	0	10	30	100	0	10	30	100
Number of animals examined	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*
Home cage	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Removal from cage	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	1
In the arena	0	0	0	0	1	0	0	0	5	4	8	6	4	3	5	7*

From Hughes (1997b)
* $P = 0.007$

On day 7, the incidence of defecation was statistically significantly increased among males at 100 mg/kg bw. In the absence of other effects, this change was not considered attributable to treatment. On day 14, there was no remarkable difference between the groups.

There were no statistically significant differences in activity counts or rearing counts between the observation days.

Six hours after dosing, there were statistically significantly increased values recorded for forelimb grip strength among males treated at 100 mg/kg bw. Forelimb grip strength among females at 100 mg/kg bw was slightly increased from controls, but differences failed to attain statistical significance. There were no statistically significant differences at lower doses. Hindlimb grip strength was also increased among males treated at 100 mg/kg bw, with differences just failing to attain statistical significance. Among females, values were comparable between treated and control groups. There were no statistically significant differences at lower doses 6 hours after dosing as well as on day 7 or 14 (Tables 64 and 65).

Only 5 hours after dosing, there was a tendency for lower splay values among males treated at 100 mg/kg bw. Among females treated at 100 mg/kg bw, mean splay values were statistically significantly lower compared with controls (Table 66). At the same time, the body temperature values of males and females of the same group were statistically significantly lower than those of the controls (Table 67). At lower doses, values were considered comparable with those of controls. There was no statistically significant difference on days 7 and 14.

Six hours after dosing, Coulbourn activity was statistically significantly lower among males treated at 30 and 100 mg/kg bw and among females treated at 100 mg/kg bw. At 100 mg/kg bw, there was basically no activity. For males at 30 mg/kg bw, the initial level of activity was lower compared with controls (Table 68). The pattern of activity over time was similar to controls. There were no statistically significant differences in activity between treated and control groups on day 7 or 14.

There were no microscopic findings that were considered to be related to the administration of acetamiprid.

In view of the above findings, it can be concluded that a single acute dose of acetamiprid at 10, 30 or 100 mg/kg bw was associated with clinical signs of decreased weight gain and lower feed consumption among males treated at 100 mg/kg bw during the week following treatment.

Behavioural changes were confined to the day of treatment. Six hours after an acute dose of acetamiprid, a range of effects was observed, particularly at 100 mg/kg bw. Behavioural observations

Table 64. Group mean forelimb grip strength, day 0, 6 hours post-dosing

Dose (mg/kg bw)	Forelimb grip strength (kg)			
	Males		Females	
	Mean	Standard deviation	Mean	Standard deviation
0	0.71	0.157	0.77	0.108
10	0.81	0.085	0.75	0.165
30	0.82	0.127	0.81	0.104
100	0.94**	0.110	0.86	0.062

From Hughes (1997b)
** $P < 0.05$

Table 65. Group mean hindlimb grip strength, day 0, 6 hours post-dosing

Dose (mg/kg bw)	Mean hindlimb grip strength (kg)			
	Males		Females	
	Mean	Standard deviation	Mean	Standard deviation
0	0.64	0.151	0.75	0.149
10	0.70	0.193	069	0.177
30	0.71	0.191	0.76	0.171
100	0.81	0.174	0.73	0.115

From Hughes (1997b)

at 100 mg/kg bw included observations of tremor, dilated pupils, increased urination (among males) and altered patterns of gait and posture. There were clear effects on temperature, which was reduced. Grip strength was increased, and landing foot splay was decreased. The level of locomotor activity was reduced.

At 30 mg/kg bw, there was evidence of tremor among males (one animal showed continuous tremor), and one female showed tail tremor. There was also an indication of increased urination among males. There was a statistically significant reduction of locomotor activity among males.

At 10 mg/kg bw, there were no effects of treatment. The singular occurrence of chewing behaviour in one female in the absence of other changes was not considered clearly related to treatment.

The main effect of treatment would appear to be on the central nervous system, with the observation of tremors. There were some effects on the autonomic nervous system, as indicated by observations of increased urination and increased pupil diameter/dilation. The changes in grip strength (increased) and foot splay (decreased) would appear to be enhanced performance and are suggestive of an increase in muscle tone. There were no apparent effects on sensory systems. There was no evidence of neuropathology.

Based on these findings, the NOAEL was considered to be 10 mg/kg bw, based on evidence of increased urination frequency (males) and a statistically significant reduction of locomotor activity (males) at 30 mg/kg bw.

The study described in this report was conducted in compliance with GLP, and a QA statement was attached (Hughes, 1997b).

Subchronic neurotoxicity

Four groups of 10 male and 10 female Crl:CD BR rats were given acetamiprid (batch No. NFG-02, purity 99.9%) by dietary admixture at a level of 100, 200, 800 or 1600 ppm (equivalent to

Table 66. Group mean splay values, day 0, 6 hours post-dosing

Dose (mg/kg bw)	Splay values (cm)			
	Males		Females	
	Mean	Standard deviation	Mean	Standard deviation
0	7.9	1.74	8.6	2.11
10	9.2	2.30	8.0	1.60
30	7.5	2.02	7.2	2.30
100	6.7	1.02	6.1**	1.15

From Hughes (1997b)
** $P < 0.01$

Table 67. Group mean temperature, day 0, 6 hours post-dosing

Dose (mg/kg bw)	Mean temperature (°C)			
	Males		Females	
	Mean	Standard deviation	Mean	Standard deviation
0	37.1	0.37	37.4	0.66
10	37.3	0.44	37.5	0.58
30	37.1	0.33	37.4	0.50
100	35.4**	1.22	34.9**	2.13

From Hughes (1997b)
** $P < 0.01$

Table 68. Coulbourn activity monitoring, day 0, 6 hours post-dosing

Dose (mg/kg bw)	Mean large movements (in sets) during 1 h observation period			
	Males		Females	
	Mean	Standard deviation	Mean	Standard deviation
0	256	83.4	258	102.0
10	235	153.1	250	92.3
30	154*	40.5	219	66.6
100	45**	54.2	33**	25.2

From Hughes (1997b)
* $P < 0.05$; ** $P < 0.01$

7.4, 14.8, 59.7 and 118 mg/kg bw per day for males and 8.5, 16.3, 67.6 and 134 mg/kg bw per day for females, respectively) for 13 weeks. Dietary inclusion levels remained constant throughout the study. A similar sized group was given untreated diet and acted as controls.

All animals were subjected to a functional observational battery prior to treatment and after 4, 8 and 13 weeks of treatment. The motor activity of each animal was also quantitatively assessed at the same intervals. Throughout the study, clinical signs, body weights and feed consumption were monitored. At the end of the treatment period, all animals were killed using whole-body perfusion. Examination was confined to designated tissues of the nervous system, which were subsequently examined microscopically.

There were no mortalities. There were no clinical signs associated with treatment. Body weights at 800 and 1600 ppm, for males and females, were statistically significantly lower than those of controls. At 800 ppm, body weight gains were 80% and 66% of control values for males and females, respectively. At 1600 ppm, gains were 56% and 51% of control values for males and females, respectively (Table 69). The cumulative intake of food was statistically significantly lower among males and females treated at 800 or 1600 ppm. At 800 ppm, cumulative values were 89% and 87% of control values for males and females, respectively. At 1600 ppm, cumulative values were 77% and 81% of control values for males and females, respectively (Table 70). Utilization of feed was impaired for males at 1600 ppm and for females at 800 and 1600 ppm.

There were no changes that were considered indicative of neurotoxicity. Quantitative assessment of locomotor activity showed that it was unaffected by treatment. There were no differences in brain parameters between the groups. There were no findings in neuropathology that were attributable to treatment.

In view of the above, it can be concluded that the treatment with acetamiprid for 13 weeks at dietary levels of 100, 200, 800 or 1600 ppm was associated with effects that were limited to 800 and 1600 ppm. At 800 and 1600 ppm, treatment was associated with lower body weights and lower feed consumption values. Feed efficiency was also affected, with impaired efficiency observed at 1600 ppm for males and 800 and 1600 ppm for females. There were no behavioural changes that were considered to be indicative of neurotoxicity, nor were there any neuropathological findings that were attributed to treatment.

The NOAEL was established as 200 ppm (equivalent to 14.8 mg/kg bw per day for males and 16.3 mg/kg bw per day for females), on the basis of lower body weights and feed consumption at the next higher dose.

The study was compliant with GLP, and a QA statement was attached (Hughes, 1997c).

Developmental neurotoxicity

A range-finding study was designed with the objective to provide acetamiprid dose selection information for a developmental neurotoxicity study and to define levels inducing toxic effects from maternal exposure during organogenesis on pregnancy, parturition and lactation and on the growth, viability and development of the F_1 neonates.

Acetamiprid (batch No. NNI-03, purity 100%) was administered orally by gavage to four groups of 12 bred Crl:CD(SD)IGS BR rats (84 days old at the initiation of administration, a minimum weight of 220 g) once daily from gestation day 6 through lactation day 10, inclusively. Dose levels were 5, 10, 20 and 40 mg/kg bw per day administered at a dose volume of 5 ml/kg bw. For comparative purposes, a concurrent control group of identical design received the vehicle, 5% gum arabic with 0.01% Tween 80, on a comparable regimen at 5 ml/kg bw. All animals were observed twice daily for appearance and behaviour. Maternal body weights and feed consumption were recorded on gestation days 0, 3, 6, 9, 12, 15 and 20 as well as on lactation days 1, 3, 7, 10, 16 and 21. All of the F_0 females were allowed to deliver and rear their pups to weaning (lactation day 21). The offspring were potentially exposed to the test article in utero (placental transfer) and through nursing during lactation. F_0 females that failed to deliver were necropsied on post-mating day 25. All surviving maternal animals were euthanized on lactation day 21 and subjected to a gross necropsy.

All females survived to the scheduled necropsy. No treatment-related clinical signs or internal findings were noted at any dose level.

In the control, 5, 10, 20 and 40 mg/kg bw per day groups, 11, 12, 11, 12 and 12 females, respectively, delivered litters. The duration of gestation was unaffected by treatment at all dose levels. Mean body weight gains in the 20 and 40 mg/kg bw per day groups were reduced during gestation days 6–9 and 9–12 compared with the control group. Mean body weight gain in the 40 mg/kg bw per day group was slightly reduced compared with the control group during gestation days 15–20. When

Table 69. Body weight gain

Weeks	Body weight gain (g)									
	Males					Females				
	Dietary concentration (ppm)									
	Control	100	200	800	1600	Control	100	200	800	1600
0–4	166	156	148	120**	74**	79	75	77	50**	35**
0–8	261	246	236	202**	139**	120	116	114	80**	59**
0–13	333	327	313	265**	186**	152	144	139	100**	78**
SD										
0–4	18.3	24.9	23.3	23.4	15.6	21.8	12.3	9.9	9.0	9.5
0–8	23.9	34.5	31.8	34.0	29.2	25.2	17.0	17.1	15.3	16.0
0–13	25.5	44.4	40.0	41.0	32.1	39.3	19.9	22.7	12.6	19.7
% of control										
0–4	—	94	89	72	45	—	95	97	63	44
0–8	—	94	90	77	53	—	97	95	67	49
0–13	—	98	94	80	56	—	95	91	66	51

From Hughes (1997c)
SD, standard deviation; ** $P < 0.01$

Table 70. Cumulative feed consumption, group mean values

	Cumulative feed consumption (g)									
	Males					Females				
	Dietary concentration (ppm)									
	Control	100	200	800	1600	Control	100	200	800	1600
Weeks 1–13	2801	2719	2718	2493**	2146**	2039	2014	1944	1782**	1659**
SD	132.1	209.9	133.5	173.7	126.1	237.9	168.4	105.1	143.7	217.1
% of control	—	97	97	89	77	—	99	95	87	81

From Hughes (1997c)
SD, standard deviation; ** $P < 0.01$

the entire gestational treatment period (gestation days 6–20) was evaluated, mean body weight gains in the 20 and 40 mg/kg bw per day groups were reduced (7.8% and 17%, respectively) compared with the control group. Mean body weights in the 20 mg/kg bw per day group were similar to those of the control group throughout gestation and lactation; however, the 40 mg/kg bw per day group values were slightly reduced during gestation days 12, 15 and 20 and throughout lactation (lactation days 1, 3, 7, 10, 16 and 21). Mean body weight gains in the 20 and 40 mg/kg bw per day groups were unaffected by treatment during lactation. Mean body weights and body weight gains in the 5 and 10 mg/kg bw per day groups were unaffected by treatment during gestation and lactation.

In the 40 mg/kg bw per day group, feed consumption was reduced relative to the control group for gestation days 6–9, 9–12, 12–15 and 15–20 and when the entire gestational treatment period (gestation days 6–20) was evaluated. In the 20 mg/kg bw per day group, feed consumption was reduced during gestation days 6–9. When the entire gestational treatment period (gestation days 6–20) was evaluated, feed consumption in the 20 mg/kg bw per day group was reduced compared with the control group. Feed consumption in the 20 and 40 mg/kg bw per day groups was unaffected by treatment

during lactation. Feed consumption in the 5 and 10 mg/kg bw per day group was unaffected by treatment throughout the study. The mean numbers of pups born, mean live litter size and percentage of males per litter at birth were unaffected by treatment in the 5, 10, 20 and 40 mg/kg bw per day groups. Postnatal survival in the treated groups was similar to that in the control group throughout the postnatal period. The general physical condition of the F_1 pups in the 5, 10, 20 and 40 mg/kg bw per day groups was similar to that of the control group. Mean pup weights in the 40 mg/kg bw per day group were reduced relative to the control group values during postnatal days 4 (pre- and post-selection), 7 and 10. Mean pup weights in the 5, 10 and 20 mg/kg bw per day groups were generally similar to the control group values throughout the postnatal period. The general physical condition of the F_1 pups during the postnatal period was unaffected by treatment at all dose levels. Necropsy findings for pups that were found dead or at the scheduled euthanasia on postnatal day 21 were not suggestive of any correlation with maternal treatment.

In conclusion, there were no observations indicative of neurotoxicity in either the maternal animals or the F_1 offspring. Maternal toxicity, however, was exhibited at dose levels of 20 and 40 mg/kg bw per day as reductions in mean body weight gain and feed consumption. Gestation length was unaffected by treatment at all dose levels. Neonatal toxicity was exhibited in the 40 mg/kg bw per day group as reduced mean pup weights. Based on the results of this study, dose levels of 2.5, 10 and 45 mg/kg bw per day were selected for a developmental neurotoxicity study of acetamiprid in rats.

This study was conducted in compliance with GLP (Nemec, 2004).

An oral developmental toxicity study was designed to determine the potential of the test article, acetamiprid, to induce functional and/or morphological changes in the nervous system, which may arise in the offspring from exposure of the mother during pregnancy and lactation. Acetamiprid (lot No. NNI-03, purity > 99%) was administered orally by gavage to three groups of 25 bred Crl:CD(SD) IGS BR rats (75 days old at the initiation of the study, weight 210–270 g) once daily from gestation day 6 through lactation day 21, inclusively. Dose levels were 2.5, 10 and 45 mg/kg bw per day administered at a dose volume of 5 ml/kg bw. For comparative purposes, a concurrent control group of identical design received the vehicle, 5% gum arabic with 0.01% Tween 80, on a comparable regimen at 5 ml/kg bw.

All animals were observed twice daily for appearance and behaviour. Clinical observations were performed on all females daily. In addition, detailed clinical observations were performed on 10 females per group on gestation days 6 and 12 and on lactation days 4 and 7. Maternal body weights and feed consumption were recorded on gestation days 0, 3, 6, 9, 12, 15 and 20, as well as on lactation days 1, 4, 7, 10, 16 and 21. All of the F_0 females were allowed to deliver and rear their pups to weaning (lactation day 21). The offspring were potentially exposed to the test article in utero (placental transfer) and through nursing during lactation. F_0 females that failed to deliver were necropsied on post-mating day 25. F_0 females that delivered were allowed to rear their offspring to lactation day 21 and were then necropsied. F_0 females with litters that did not meet the sex ratio criteria were necropsied with their litters on postnatal day 4. Following culling on postnatal day 4, 10 pups of each sex per group were randomly assigned to one of the following tests: learning and memory (different pups for each interval), locomotor activity and acoustic startle. Additional pups were assigned to neuropathological, brain weight and brain morphometric evaluations on postnatal day 11 (one pup of each sex per litter) and postnatal day 72 (10 pups of each sex per group). Detailed clinical observations were performed on 10 pups of each sex per group on postnatal days 4, 11, 21, 35, 45 and 60. Indicators of physical development (balanopreputial separation and vaginal patency) were evaluated for all pups. All pups not selected for behavioural evaluations were euthanized and necropsied on postnatal day 28.

One female in the 45 mg/kg bw per day group died during parturition on gestation day 23, following delivery of one pup. This dystocia-related death was considered potentially treatment related.

All other females survived to the scheduled necropsies. No adverse clinical signs of toxicity were noted. Pregnancy rates were 92.0%, 92.0%, 92.0% and 100% in the control, 2.5, 10 and 45 mg/kg bw per day groups, respectively. A mean body weight loss was observed in the 45 mg/kg bw per day group during the first 3 days of dosing (gestation days 6–9). Mean gestation body weights in this group were lower than the control group values during the majority of gestation. Mean body weight in this group was reduced compared with the control group on lactation day 1. Mean lactation body weight gain in the 45 mg/kg bw per day group was increased on lactation days 1–4. Mean gestation and lactation body weights in the 2.5 and 10 mg/kg bw per day groups were similar to the control group values.

Feed consumption in the 45 mg/kg bw per day group was reduced during gestation days 6–9 and 9–12. During lactation, feed consumption in this group was similar to that in the control group. Feed consumption in the 2.5 and 10 mg/kg bw per day groups was similar to the control group values during gestation and lactation.

No treatment-related internal findings were noted at the scheduled necropsies of the F_0 females. The mean numbers of implantation sites and sites that were unaccounted for in the treated groups were similar to those in the control group. Mean live litter size in the 45 mg/kg bw per day group was 7.3% lower than the control group value. Mean live litter size in the 2.5 and 10 mg/kg bw per day groups was similar to that in the control group. Postnatal survival in the 45 mg/kg bw per day group was reduced on postnatal day 0 and postnatal days 0–1. Three females in this group had total litter loss on postnatal day 1. Postnatal survival in the 2.5 and 10 mg/kg bw per day groups was not affected by F_0 maternal test article administration.

The general physical condition of the F_1 animals during the pre-weaning and post-weaning periods was similar in all groups, including the control group. Mean offspring body weight gains in the treated groups were similar to the control group values during the pre-weaning period. Mean body weights in the 45 mg/kg bw per day group males and females, however, were statistically significantly reduced on postnatal day 1 and were numerically reduced throughout the remainder of the pre-weaning period. Mean body weight gains in the 45 mg/kg bw per day group males were reduced during postnatal days 35–72. Mean body weight gains in the 45 mg/kg bw per day group females were reduced on postnatal days 28–35 and 49–56. Mean body weights of these males and females were reduced throughout the post-weaning period. Mean body weights and body weight gains in the 2.5 and 10 mg/kg bw per day groups were not affected by F_0 maternal test article administration. The mean numbers of days to acquisition of balanopreputial separation and vaginal patency in the treated group males and females were not affected by F_0 maternal test article administration. Mean body weights in the treated groups on the day of acquisition of balanopreputial separation were similar to the control group value. Mean body weight in the 45 mg/kg bw per day group females was reduced on the day of vaginal patency acquisition. Mean body weights on the day of acquisition of vaginal patency in the 2.5 and 10 mg/kg bw per day group females were similar to the control group value.

For auditory startle response, the maximum response amplitudes (V_{max}) and average response amplitudes (V_{avg}) were reduced on postnatal days 20 and 60 in the 45 mg/kg bw per day group males. V_{max} and V_{avg} were reduced in the 45 mg/kg bw per day group females on postnatal day 20. V_{max} and V_{avg} in the 45 mg/kg bw per day group females were similar to the control group values on postnatal day 60. Auditory startle response in the 2.5 and 10 mg/kg bw per day group males and females was unaffected by F_0 maternal test article administration.

Locomotor activity and learning and memory in the 2.5, 10 and 45 mg/kg bw per day group offspring were unaffected by F_0 maternal test article administration. Necropsy findings for F_1 pups that were found dead, euthanized in extremis and euthanized due to failure to meet sex ratio criteria during the pre-weaning period were not suggestive of any correlation with maternal treatment. At the scheduled necropsy of pups that were not selected for behavioural evaluations (postnatal day 28), the postnatal day 72 necropsies of offspring not selected for neuropathology and brain weight

measurements and the postnatal day 11 and postnatal day 72 necropsies of offspring selected for neuropathology and brain weight measurements, no internal findings were observed that could be attributed to maternal test article administration. There were no test article–related microscopic findings in the central and peripheral nervous system tissues examined from the selected postnatal day 11 and postnatal day 72 pups. Mean absolute brain weights, brain weights relative to final body weights, brain lengths, brain widths and F_1 brain morphometry at postnatal day 11 and postnatal day 72 in the treated group males and females were not affected by F_0 maternal test article administration.

In conclusion, F_0 maternal toxicity was expressed at a dose level of 45 mg/kg bw per day by a single mortality and reductions in body weight gain and feed consumption. No maternal toxicity was exhibited at dose levels of 2.5 and 10 mg/kg bw per day. F_1 developmental toxicity was expressed at a dose level of 45 mg/kg bw per day by early postnatal mortality and reduced post-weaning body weights. No developmental toxicity was exhibited at dose levels of 2.5 and 10 mg/kg bw per day.

Deficits in auditory startle response occurred in the 45 mg/kg bw per day group F_1 males and females without concomitant effects in other functional end-points (functional observational battery), neuropathology or brain morphometry. For this reason, as auditory startle response amplitude is affected by treatment, the conclusion could be that some part of the pathway is affected. The affected component could be sensory or it could be motor, but some part of the nervous system (or muscle fibre activity) is affected. However, the startle response may not be free from central involvement, as there is ample evidence for descending inhibitory activity, which may, in fact, be the basis for habituation (Tyl et al., 2008).

Based on the results of this study, the NOAEL for maternal toxicity, developmental toxicity and developmental neurotoxicity was considered to be 10 mg/kg bw per day.

This study was conducted in compliance with GLP, and a QA statement was attached (Nemec, 2003).

Acute delayed neurotoxicity

To determine the acute oral toxicity of acetamiprid in adult hens, six groups of 10 birds (hybrid brown laying strain *Gallus gallus domesticus* approximately 15 months old, weight range 1930–2295 g) were given a single dose of acetamiprid (batch No. NNI-03, purity > 99%) and observed for 14 days. The LD_{50} value was calculated to be 129 mg/kg bw.

To investigate any delayed neurotoxic effects of acetamiprid, the doses used in the neurotoxicity assessment were based on the LD_{50} value. The study involved a group of 32 birds (hybrid brown laying strain *Gallus gallus domesticus* approximately 14 months old, weight range 2090–2395 g). Negative and positive control groups were also dosed, with 12 birds allocated to each. Observations included mortality, adverse clinical signs, assessment of delayed locomotor ataxia and body weight. Forty-eight hours after dosing, predetermined birds were examined for brain acetylcholinesterase and brain and spinal cord neuropathy target esterase activities. At the end of the observation period, additional designated birds were sent for histopathological examination. During the neurotoxicity assessment, all birds treated with acetamiprid either died or showed clinical signs of toxicity, which included unsteadiness and subdued behaviour. There were no signs of delayed locomotor ataxia in any bird treated with acetamiprid. One bird in the positive control group showed slight incoordination. Brain acetylcholinesterase and brain and spinal cord neuropathy target esterase activities in birds treated with acetamiprid were considered to be normal. Oral administration of a single dose of acetamiprid at the LD_{50} dose level of 129 mg/kg bw did not produce any clinical signs of delayed neurotoxicity when assessed in terms of ataxia. There was no histological evidence of acute delayed neurotoxicity. No other treatment-related changes were detected in this examination. As expected, given the absence of any other indication of delayed neurotoxicity, there were no significant reductions in brain acetylcholinesterase levels or neuropathy target esterase levels in the brain and spinal cord.

This study was conducted in compliance with GLP, and a QA statement was attached (Redgrave, 1994).

(b) Immunotoxicity

Mice

To assess the functional responsiveness of specified elements of the immune system using a modification of the Jerne plaque-forming cell assay in the CD-1 mouse, three groups of mice, each comprising 10 males and 10 females, were administered acetamiprid (lot No. NFG-02, purity 99.9%) in the diet at a concentration of 100, 300 or 900 ppm (equal to 15.3, 47.0 and 128 mg/kg bw per day for males and 20.2, 54.1 and 157 mg/kg bw per day for females, respectively) for a period of 4 weeks. A similarly constituted control group received the vehicle, basal diet. The age of animals at the start of the study was between 47 and 56 days, and their body weights were in the range of 29.2–38.9 g for males and 22.7–33.0 g for females. A further eight males and eight females received cyclophosphamide (a positive control) at 20 mg/kg bw, administered as five daily oral (gavage) doses on days 22–26. During the study, clinical condition, body weight, feed and water consumption, organ weight, macropathology and plaque-forming cell assays were undertaken.

There were no unscheduled deaths. Noisy breathing (rales) was reported in week 4 in 2 of 10 males and 3 of 10 females receiving 900 ppm.

Body weight gains were low during the 4-week treatment period for males and females receiving 900 ppm, with the magnitude of the effect being greatest on days 1–7, when several animals experienced weight loss (Table 71).

Feed intake at 900 ppm was reduced in weeks 1 and 2, and water intake was slightly lower than that of controls throughout the treatment period in males and in week 1 in females. Females receiving 300 ppm had low body weight gain from the end of week 1 and low feed consumption in weeks 1 and 2 (Table 72).

Low spleen weights were reported after 4 weeks in females receiving 900 ppm. There were no treatment-related effects on thymus weights or macropathology.

There was considered to have been no effect of treatment on the immune system. Female mice given acetamiprid at 900 ppm showed a statistically significant reduction in the observed number of plaque-forming cells per spleen. This finding correlates with a general reduction in total cell numbers per spleen, showing that the reduced response was due to a reduction in cell number, rather than the immune function of the cells. There was no similar trend in males (Table 73).

It is concluded that dietary administration of acetamiprid to CD-1 mice at dietary concentrations up to 900 ppm for 4 weeks caused a nonspecific toxic response at 300 ppm in females and at 900 ppm in both sexes, but there was no effect on immune function, as assessed by the measurement of antigen-specific, T cell–dependent antibody formation. The NOEL for immunotoxicity by acetamiprid in mice was therefore greater than 900 ppm (i.e. > 128 mg/kg bw per day in males and > 157 mg/kg bw per day in females), the highest dose tested.

This study was conducted in compliance with GLP, and a QA statement was attached (Bown, 2010).

Rats

A study was conducted to assess the functional responsiveness of specified elements of the immune system, using a modification of the Jerne plaque-forming cell assay, in the Sprague-Dawley rat following dietary administration for 4 weeks. Three groups of Sprague-Dawley rats (Crl:CD (SD) strain), each comprising 10 males and 10 females, received acetamiprid (lot No. NFG-02, purity 99.9%) in the diet at a concentration of 100, 300 or 900 ppm (equivalent to 7.4, 21.6 and 62.9 mg/kg bw per day for males and 8.6, 24.3 and 67.7 mg/kg bw per day for females). A similarly constituted

Table 71. Summary of body weight gain

Days	Body weight gain (g)							
	Males				Females			
	Dietary concentration (ppm)							
	Control	100	300	900	Control	100	300	900
1–7	1.8	0.7	1.4	−0.3**	1.0	0.9	1.0	−0.2*
8–29	2.8	3.6	3.1	2.3	2.2	3.1	1.8	1.7
1–29	4.6	4.3	4.5	2.0**	3.2	4.0	2.8	1.5
% of control (days 1–29)	—	93	98	44	—	125	88	48

From Bown (2010)
* $P < 0.05$; ** $P < 0.01$

Table 72. Summary of feed consumption

Weeks	Feed consumption (g/week)							
	Males				Females			
	Dietary concentration (ppm)							
	Control	100	300	900	Control	100	300	900
1–2	39[a]	36	42	31**	37	38	33	31*
3–4	38	41	41	38	41	45	39	37
1–4	39[a]	38	42	34	39	41[a]	36	34

From Bown (2010)
* $P < 0.05$; ** $P < 0.01$
[a] Feed consumption value includes an estimate of a missing value.

control group received the vehicle, basal diet. A further eight males and eight females received a single intraperitoneal dose of cyclophosphamide (a positive control) at 50 mg/kg bw 2 days prior to necropsy (i.e. on day 27). The age of those animals selected for the study at the start of treatment was 48–57 days, and their body weights were in the range of 262–330 g for males and 184–232 g for females. During the study, clinical condition, body weight, feed and water consumption, organ weights, macropathology and plaque-forming cell assays were undertaken.

There were no unscheduled deaths or any treatment-related clinical signs. Body weight gains and feed consumption (Tables 74 and 75) were low during the 4-week treatment period for males and females receiving 900 ppm, with the magnitude of the effect on body weight being greatest on days 1–3 and the effect on feed consumption being greatest in weeks 1 and 2 for males. Males receiving 300 ppm had low body weight gain during days 1–3 and low feed consumption in week 1.

Water consumption was low for males receiving 300 or 900 ppm. There were no treatment-related effects on spleen and thymus weights or macropathology. There were no statistically significant changes in the numbers of cells per spleen, plaque-forming cells per 10^6 cells or plaque-forming cells per spleen for any of the groups that received acetamiprid.

It is concluded that dietary administration of acetamiprid to Sprague-Dawley rats at dietary concentrations up to 900 ppm for 4 weeks caused a nonspecific toxic response at 300 ppm in males and 900 ppm in both sexes, but there was no effect on immune function, as assessed by the measurement of antigen-specific, T cell–dependent antibody formation.

The NOEL for immunotoxicity by acetamiprid in rats was therefore greater than 900 ppm (i.e. > 62.9 mg/kg bw per day in males and > 67.7 mg/kg bw per day in females), the highest dose tested.

Table 73. Summary of immunotoxicity findings

	Dietary concentration (ppm)				Cyclophosphamide[a]
	Control	100	300	900	
Males					
Cells/spleen (× 10^7)	6.65	5.64	7.04	6.73	3.06**
PFC/10^6 cells	1611.5	1076.5	1062.5	1350.5	186.6***
PFC/spleen	104 208	59 182	78 226	101 745	8098***
Females					
Cells/spleen (× 10^7)	9.46	7.68	7.33	6.08*	4.53**
PFC/10^6 cells	1740.0	1333.8	1737.3	1525.5	320.6***
PFC/spleen	170 060	105 397	116 812	93 878*	17 073***

From Bown (2010)
PFC, plaque-forming cells
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$
[a] 20 mg/kg bw per day administered as five daily oral doses on days 22–26.

The study was conducted in compliance with GLP, and a QA statement was attached (Moore, 2010).

(c) Pharmacological studies

The general pharmacological properties of acetamiprid (lot No. NNI-02, purity 99.46%) were investigated in mice, rats, guinea-pigs and rabbits.

Animals used in this study were as follows:

- male Crj:ICR mice (5–6 weeks old, weight 27.7–31.9 g);
- male Crj:CD(SD) rats (7 weeks old, weight 262.4–323.2 g);
- male Std:Hartley guinea-pigs (4–6 weeks old, weight 284.2–416.7 g);
- male Kbs:NZW rabbits (11–13 weeks old, weight 2.48–2.84 kg).

Dosage and dose volume

Based on the results of the study on general activity and behaviour in mice (1, 3, 5, 10, 20, 30 and 60 mg/kg bw intraperitoneally), three doses (5, 10 and 20 mg/kg bw) at a common ratio of 2 were selected for the intraperitoneal experiments. Three doses (10, 20 and 40 mg/kg bw) at a common ratio of 2 were selected for the oral experiments. In the study on general activity and behaviour in rabbits, doses of 10, 30 and 60 mg/kg bw were administered. In this study using anaesthetized rabbits, doses of 1, 3 and 10 mg/kg bw were administered. In the in vitro experiment, concentrations of 10^{-6}, 10^{-5}, 10^{-4} and 10^{-3} g/ml at a common ratio of 10 were selected. The solvent used to dissolved the test substance was used for the corresponding vehicle group in each study.

Dose volumes were 10 ml/kg bw for oral and intraperitoneal administration and 3 ml/kg bw for intravenous injection in the study using conscious rabbits and 1 ml/kg bw for intravenous injection in the study using anaesthetized rabbits.

Results

Effects on general activity and behaviour. In the study of general activity and behaviour in mice, lower doses of acetamiprid (1, 3 and 5 mg/kg bw) (intraperitoneal) showed no clinical signs. One out

Table 74. Summary of body weight gain

	Body weight gain (g)							
	Males				Females			
	Dietary concentration (ppm)							
	0	100	300	900	0	100	300	900
Days 1–3	23	21	17*	1**	4	7	4	−2**
Days 4–29	125	122	120	98**	44	37	42	25**
Days 1–29	148	144	137	99**	48	44	47	23**
% of control (days 1–29)	—	97	93	67		92	97	48

From Moore (2010)
* $P < 0.05$; ** $P < 0.01$

Table 75. Summary of feed consumption

	Feed consumption (g/week)							
	Males				Females			
	Dietary concentration (ppm)							
	0	100	300	900	0	100	300	900
Weeks 1–2	199	192	177	158	135	142	129	115
Weeks 3–4	194	193	186	174	140	137	138	120
Weeks 1–4	196	192	181	166	137	139	133	117

From Moore (2010)

of the three mice at 10 mg/kg bw showed vocalization and slight depression of spontaneous motor activity. All mice of the 20 mg/kg bw group showed the following signs: a slight to moderate decrease in alertness, grooming, reactivity and spontaneous activity, a slight decrease in grip strength, tremor, and abnormality of body and limb position. At 30 mg/kg bw, a slight decrease in alertness, grooming, touch response, pain response, passivity righting reflex, limb tone, pinna reflex and ipsilateral flexor reflex, a severe decrease in reactivity, spontaneous activity and grip strength, an abnormality of body position, tremor and convulsion were observed. One out of three mice died within 120 minutes of the administration. At 60 mg/kg bw, all mice showed a severe decrease in reactivity and spontaneous activity, passivity, tremor, etc. and died within 30 minutes of the administration.

In the study of general activity and behaviour in rabbits, no clinical signs were noted in the vehicle control and the 10 mg/kg bw group (intravenous). At 30 mg/kg bw, one or two out of the three rabbits showed a slight decrease in spontaneous activity, alertness, limb tone, abdominal tone and papillary reflex, a slight increase in respiratory rate and convulsion, motor incoordination and mydriasis from 5 to 180 minutes after the administration. All of these clinical signs disappeared 1 day after the administration. At 60 mg/kg bw, the rabbits showed a severe decrease in spontaneous activity and muscle tone, convulsion, mydriasis, abnormal respiration and cyanosis, etc., and all animals died within 60 minutes of the administration.

Effects on the central nervous system. The test substance elicited no obvious effects on anticonvulsant activity and pain response in mice and on body temperature in rats in the range of 5–20 mg/kg bw (intraperitoneal). In contrast, decreases in motor activity and hypnotic prolongation action were recognized with a dose of 20 mg/kg bw (intraperitoneal).

Effects on the somatic nervous system. No muscle relaxation effects in mice were observed at 5 or 10 mg/kg bw (intraperitoneal). At 20 mg/kg bw, muscle tone tended to be reduced 60–90 minutes after the injection, without statistical significance.

Effects on the autonomic nervous system and smooth muscle (isolated guinea-pig ileum). No direct action was noted at 10^{-6} or 10^{-5} g/ml of the test substance. However, at higher doses (10^{-4} and 10^{-3} g/ml), a transient contraction followed by relaxation was noted. The contraction caused by each agonist was not affected by the test substance (10^{-6} to 10^{-4} g/ml).

At 10^{-3} g/ml, the test substance significantly inhibited the contraction caused by each agonist.

Effects on the respiratory and cardiovascular systems (rabbits). At 1 mg/kg bw, no significant effect was noted on the parameters determined. At 3 and 10 mg/kg bw, a decrease in blood pressure and a slight and transient increase in respiratory rate were noted. The heart rate was not altered by the treatment at any dose.

Effects on the digestive system (mice). Gastrointestinal transport was not affected at 10 or 20 mg/kg bw. However, a statistically significant decrease in gastrointestinal transport was noted at 40 mg/kg bw.

Effects on water and electrolyte metabolism (rats). At 5 and 10 mg/kg bw, the test substance did not alter the urine volume or urinary sodium, potassium and chloride concentrations. However, a statistically significant decrease in urine volume and urinary sodium and chloride concentrations was noted at 20 mg/kg bw.

Effects on blood (rats). No significant changes were noted in blood clotting or haemolysis in rats at 5–20 mg/kg bw.

Effects on plasma cholinesterase activity. Plasma cholinesterase activity was not altered by the intraperitoneal administration of acetamiprid (5–20 mg/kg bw) in rats.

Summary. From the experiments described above, the effect levels of acetamiprid for intraperitoneal, intravenous and oral administration and in vitro tests were 10 mg/kg bw, 3 mg/kg bw, 40 mg/kg bw and 10 g/ml, respectively.

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

(d) Studies on impurities

The results of five batch analyses submitted are shown in Table 76.

From Table 76, it can be seen that the impurities present in acetamiprid are AM-1, AM-2, AM-3 and AM-4. The toxicological studies on these impurities that have been submitted are summarized in Tables 77 and 78. All studies were GLP compliant.

(e) Toxicity of metabolites

There are nine compounds identified as plant metabolites. These are IM-1-3, IM-1-4, IM-2-1, IM-2-3, IM-2-4, IM-O, IC-O, IS-1-1 and IS-2-1. The acute toxicity and genotoxicity of these compounds are summarized in Tables 79 and 80. Subchronic toxicity studies on IM-1-4 and IM-O are also described. All these studies were GLP compliant.

Subchronic toxicity study on IM-1-4

The subchronic toxicity of IM-l-4 administered in the diet to Sprague-Dawley Crl:CD BR rats for 13 weeks was evaluated in this study. Male and female rats were assigned to five groups (10 of each sex per group). Each group received IM-1-4 in the diet at the following concentrations: 0, 200, 600, 1800 or 5400 ppm (equal to 0, 12.8, 36.5, 112.2 and 319.3 mg/kg bw per day for males and 0, 15.6, 44.6, 135.6 and 356.1 mg/kg bw per day for females, respectively; however, the compound intake corresponding to 5400 ppm for females related to mean intake between 8 and 13 weeks, as the week 6 feed consumption data were lost for this group). At the initiation of dosing, the animals were approximately 6 weeks old, with body weights ranging from 211 to 270 g for the males and from 154 to 192 g for the females.

Table 76. Analysis of impurities

Lot No.	Contents (% w/w)						
	Acetamiprid	Impurities				Volatiles	Total
		AM-1	AM-2	AM-3	AM-4		
NJLP-18	100.1	0.01	0.05	0.05	0.06	0.1	100.3
NJLP-26	100.0	0.01	0.05	0.04	0.06	< 0.1	100.2
NAAP-24	99.9	0.03	0.05	0.05	0.06	< 0.1	100.1
NAAP-29	100.1	0.03	0.06	0.06	0.06	< 0.1	100.3
NABP-08	100.0	0.03	0.05	0.04	0.06	< 0.1	100.1
Minimum	99.9	0.01	0.05	0.04	0.06	< 0.1	100.1
Maximum	100.1	0.03	0.06	0.06	0.06	0.1	100.3
Mean	100.01	0.02	0.05	0.05	0.06	—	100.2
Standard deviation	0.09	0.01	0.00	0.01	0.00	—	—
Mean + 3 × standard deviation	99.56	0.05	0.07	0.08	0.06	—	—
Specification	99.0	0.2	0.2	0.2	0.2	0.5	—

From five batch analysis reports submitted to WHO
w/w, weight per weight

Table 77. Acute toxicities of impurities

Impurity	Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	Reference
AM-1	Rat	Crj:CD(SD)	Male	Oral	> 5000	Mochizuki (1994b)
			Female	Oral	4811	
AM-2	Rat	Crj:CD(SD)	Male	Oral	603	Mochizuki (1994c)
			Female	Oral	806	
AM-3	Rat	Crj:CD(SD)	Male	Oral	2543	Mochizuki & Goto (1994d)
(=IM-2-1)			Female	Oral	1762	
AM-4	Rat	Crj:CD(SD)	Male	Oral	924	Mochizuki (1994d)
			Female	Oral	1121	

LD_{50}, median lethal dose

Table 78. Results of genotoxicity studies on impurities

Impurity	End-point	Test object	Concentration (µg/plate)	Result	References
AM-1	Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 *Escherichia coli* WP2*uvrA*	313, 625, 1250, 2500 5000	Negative both with and without metabolic activation	Kanaguchi (1994a)
AM-2	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 *E. coli* WP2*uvrA*	313, 625, 1250, 2500 5000	Negative both with and without metabolic activation	Kanaguchi (1994b)
AM-3 (=IM-2-1)	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 *E. coli* WP2*uvrA*	313, 625, 1250, 2500 5000	Negative both with and without metabolic activation	Mochizuki & Kanaguchi (1994f)
AM-4	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 *E. coli* WP2*uvrA*	313, 625, 1250, 2500 5000	Negative both with and without metabolic activation	Kanaguchi (1994c)

Table 79. Results of acute toxicity studies on plant metabolites

Plant metabolites	Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	Reference
IM-1-3	Rat	Crj:CD(SD)	Male	Oral	1142	Mochizuki & Goto (1994b)
			Female	Oral	900–1000	
IM-1-4	Rat	Crj:CD BD	Male	Oral	1223.7	Wakefield (1998b)
			Female	Oral	962.84	
	Rat	Crj:CD(SD)	Male	Oral	1259	Mochizuki & Goto (1994c)
			Female	Oral	1176	
	Rat	Crj:CD BD	Male	Dermal	> 2000	Wakefield (1998a)
			Female	Dermal	> 2000	
IM-2-1 (=AM-3)	Rat	Crj:CD(SD)	Male	Oral	2543	Mochizuki & Goto (1994d)
			Female	Oral	1762	
IM-2-3	Rat	Crj:CD(SD)	Male	Oral	1378	Mochizuki & Goto (1994e)
			Female	Oral	900–1000	
IM-2-4	Rat	Crj:CD(SD)	Male	Oral	1592	Mochizuki (1994e)
			Female	Oral	1381	
IM-O	Rat	Crj:CD(SD)	Male	Oral	1842	Mochizuki & Goto (1993d)
			Female	Oral	1483	
IC-O	Rat	Crj:CD(SD)	Male	Oral	> 5000	Mochizuki & Goto (1993c)
			Female	Oral	> 5000	
IS-1-1	Rat	Crj:CD(SD)	Male	Oral	2662	Mochizuki & Goto (1994f)
			Female	Oral	2420	
IS-2-1	Rat	Crj:CD(SD)	Male	Oral	> 5000	Mochizuki & Goto (1994g)
			Female	Oral	> 5000	

LD_{50}, median lethal dose

Diet and water were provided ad libitum. The animals were observed twice daily (morning and afternoon) for mortality and moribundity, and daily cage-side observations (cage was opened) were performed for obvious indications of a toxic effect. At least once each week, each animal was removed from its cage and examined for abnormalities and signs of toxicity. Body weights and feed consumption data were collected weekly.

Ophthalmic examinations were conducted prior to treatment and during week 13. Blood and urine samples were collected for haematology and coagulation, serum chemistry and urinalysis tests from all animals prior to termination. At necropsy, macroscopic observations were recorded, selected organs were weighed and selected tissues were collected and preserved. Microscopic examinations were done on tissues from each animal in the control and high-dose groups. The lungs, liver, kidney, spleen and gross lesions were also examined microscopically from each animal in the low- and mid-dose groups.

M-l-4 consumption (milligrams per kilogram body weight per day) was greater in the females than in the males at all dose levels for the duration of the study. There were no compound-related deaths, clinical or macroscopic pathology findings or effects on ophthalmology, haematology and coagulation, urinalysis or organ weights. Significant compound effects on mean feed consumption and body weights that were likely related to reduced diet palatability were observed in the males and females that received the 5400 ppm diet. In the males, mean feed consumption was also significantly reduced below the controls during multiple weeks at both the 600 and 1800 ppm dose levels, although a corresponding significant reduction in body weight was not observed. Female mean body weight

Table 80. Results of genotoxicity studies on plant metabolites

Plant metabolites	End-point	Test object	Concentration	Result	References
IM-1-3	Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 *Escherichia coli* WP2*uvrA*	313, 625, 1250, 2500, 5000 µg/plate	Negative both with and without metabolic activation	Mochizuki & Kanaguchi (1994d)
IM-1-4	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 *E. coli* WP2*uvrA*	313, 625, 1250, 2500, 5000 µg/plate	Negative both with and without metabolic activation	Mochizuki & Kanaguchi (1994e)
	Forward mutation assay	*HGPRT* locus in Chinese hamster ovary cells	250, 500, 1000, 2000, 2500, 3000 µg/ml	Negative both with and without metabolic activation	Cifone (1998)
	In vivo mouse micronucleus assay	Crl:CD-1(ICR)BR mouse bone marrow	175, 350, 700 mg/kg bw	Negative	Curry (1998)
IM-2-1 (=AM-3)	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 *E. coli* WP2*uvrA*	313, 625, 1250, 2500, 5000 µg/plate	Negative both with and without metabolic activation	Mochizuki & Kanaguchi (1994f)
IM-2-3	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 *E. coli* WP2*uvrA*	313, 625, 1250, 2500, 5000 µg/plate	Negative both with and without metabolic activation	Mochizuki & Kanaguchi (1994g)
IM-2-4	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 *E. coli* WP2*uvrA*	313, 625, 1250, 2500, 5000 µg/plate	Negative both with and without metabolic activation	Kanaguchi (1993b)
IM-O	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 *E. coli* WP2*uvrA*	313, 625, 1250, 2500, 5000 µg/plate	Negative both with and without metabolic activation	Mochizuki & Kanaguchi (1994b)
	Chromosomal aberration	Chinese hamster lung cells	*Direct method*: 1, 1.5, 2, 3 mg/ml (24 h) 0.6, 0.8, 1, 1–2 mg/ml (48 h) *Metabolic activation method*: 2, 3, 4, 5 mg/ml	Clastogenic	Kanaguchi (1994d)
	In vivo micronucleus	CD-1(ICR) mice	325, 650, 1300 mg/kg bw	Negative	Murli (1994b)

Table 80 (continued)

Plant metabolites	End-point	Test object	Concentration	Result	References
IC-O	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 *E. coli* WP2*uvrA*	313, 625, 1250, 2500, 5000 µg/plate	Negative both with and without metabolic activation	Mochizuki & Kanaguchi (1994a)
IS-1-1	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 *E. coli* WP2*uvrA*	313, 625, 1250, 2500, 5000 µg/plate	Negative both with and without metabolic activation	Mochizuki & Kanaguchi (1994h)
IS-2-1	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 *E. coli* WP2*uvrA*	313, 625, 1250, 2500, 5000 µg/plate	Negative both with and without metabolic activation	Mochizuki & Kanaguchi (1994i)

and total feed consumption were not significantly affected at the 200–1800 ppm doses. The slight reduction in total protein and globulin in the high-dose animals and the slight changes in several urinalysis parameters in the high-dose females are considered to be related to the observed decreases in body weight and feed consumption in these animals. IM-l-4-related effects were observed in the spleen (increased pigment in the splenic sinusoids) of treated males at 1800 and 5400 ppm and in the spleen of treated females at 5400 ppm.

Based on the results of this study, the NOAEL is considered to be 600 ppm (equal to 36.5 mg/kg bw per day) for males and 1800 ppm (equal to 135.6 mg/kg bw per day) for females.

This study was conducted in compliance with GLP, and a QA statement was attached (Ivett, 1999).

Subchronic toxicity study on IM-O

This study was conducted to assess the subchronic toxicity of compound IM-O in Crj:CD(SD) rats. The test compound (lot No. NK-3266(Tox-563), purity 98.94%) was offered in the diet to 100 rats (10 of each sex per group) at a dose level of 0, 160, 800, 4000 or 20 000 ppm (equal to 0, 9.9, 48.9, 250.1 and 1246.6 mg/kg bw per day in males and 0, 11.1, 55.9, 275.9 and 1173.7 mg/kg bw per day in females) for a period of 13 weeks. The rats used were about 6 weeks old and weighed 108.5–207.3 g for males and 137.2–163.9 g for females, respectively. The results and conclusions are as follows:

1. *Clinical observation and mortality*: There were no signs of reaction to treatment in any treated groups of animals. All animals survived throughout the study.
2. *Body weight and feed consumption*: Mean body weights for the highest-dose (20 000 ppm) males and females were significantly less than those of the respective control groups throughout the study. Mean body weights of the high-dose groups were 78% of control values in males and 77% of control values in females. Mean body weight gains of the high-dose groups were 67% of control values in males and 57% of control values in females. Feed consumption values of these groups were lower than control values at examinations at weeks 1–4, 6, 9 and 13 (days 7–28, 42, 63 and 91) in males and at all weeks in females. Feed efficiency values of the 20 000 ppm animals were significantly decreased at weeks 1 and 10 (days 7 and 70) in males and at week 1 (day 7) in females.
3. *Ophthalmological examinations*: No effects of test compound treatment were evident at the week 12 examinations.
4. *Haematology*: No effects of test compound treatment were evident in the treated groups of animals at study termination.
5. *Biochemistry*: The statistically significant increases in serum alkaline phosphatase activity were seen only in the 20 000 ppm group females at study termination.
6. *Urinalysis*: No effects of test compound treatment were evident in the treated groups of animals at the week 13 examinations.
7. *Organ weights*: Statistically significant decreases in mean absolute weights of lung (males only) and liver (males only) and statistically significant increases in mean relative organ weight ratios of brain (both sexes), lung (females only), liver (females only), kidney (both sexes) and testis (right side only) were seen only in the 20 000 ppm groups. However, these changes were attributed to the decreased body weights in these groups (reduction rates, 21% in males, 22% in females).
8. *Necropsy and microscopic observations*: Necropsy revealed no compound-related lesions. Histologically, dose-related eosinophilic intranuclear inclusions were seen in the proximal tubular epithelium of kidneys for 20 000 ppm males and females and 4000 ppm males. Other microscopic changes were occasionally seen in the control and treated groups, but they were unrelated to the treatment of test compound.

Based on the results mentioned above, the effects of the test compound (IM-O), when offered in the diet to Crj:CD(SD) rats for 13 weeks, were decreased body weight gains, decreased feed consumption values, increased serum alkaline phosphatase activity and eosinophilic intranuclear inclusions in the proximal tubular epithelium of kidney. The NOAEL is 800 ppm (48.9 mg/kg bw per day) in males and 4000 ppm (275.9 mg/kg bw per day) in females.

This study was conducted in compliance with GLP, and a QA statement was attached (Nukui & Ikeyama, 1997).

Toxicity of other metabolites

The acute toxicity and genotoxicity of other metabolites of acetamiprid are summarized in Tables 81 and 82, respectively.

3. Observations in humans

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

3.1 Medical surveillance of manufacturing plant personnel

Nippon Soda started the commercial production of the technical material of acetamiprid at its Nihongi plant in Japan in April 1995. The workers were also engaged in the production of other chemicals. The production of acetamiprid was carried out from 8 to 30 April 1996, 7 to 17 May 1996, 28 June to 21 July 1996, 19 August to 20 September 1996 and 28 February to 10 April 1997.

During the production of acetamiprid, the following protective measures were taken to avoid exposures to chemicals and to ensure physical safety: helmet, safety goggles, safety shoes, filter respirator and rubber gloves.

Once a year, a health examination was performed on all employees in the plant. This health examination was compulsory under the Japanese occupational safety and health law regulations. The health examination consisted of physical examination, haematology, urinalysis and blood chemistry. Haematology includes haemoglobin (g/dl), haematocrit (%), erythrocytes and white blood cells. Urinalysis includes protein (mg/dl), glucose (mg/dl) and urobilinogen (mg/dl). Blood chemistry includes aspartate aminotransferase and alanine aminotransferase activities (IU/l), alkaline phosphatase activity, gamma-glutamyltransferase and total cholesterol measurements.

The data from this annual health examination on all employees involved in acetamiprid production have been presented to the sponsor. The individual data showed that although the health examination had been performed only twice since the commercial production was started in 1995, there were no significant changes in the parameters examined.

In addition to the clinical observations during the production of the technical material, there were no reports of any acute poisoning from exposure or any skin or eye irritation (Takashiba, 1997).

3.2 Direct observation (e.g. clinical cases and poisoning incidents)

As the toxicity of acetamiprid to insect pests is higher than that to humans, cases of acetamiprid poisoning are rare. However, Todani et al. (2008) reported a case of acute acetamiprid poisoning and measured the blood concentration of acetamiprid. A 79-year-old man had ingested acetamiprid and received medical attention 2 hours after ingestion. On arrival, he had consciousness disturbance (GCS-8), hypotension, nausea, vomiting and hyperglycaemia, but he had neither constricted pupils nor mucous supersecretions, which are characteristic of organophosphate poisoning. Gastric lavage was performed, and activated charcoal and laxative were administered. Paroxysmal atrial fibrillation persisted until 11

Table 81. Acute toxicity of other metabolites

Metabolite	Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	Reference
IM-1-5	Rat	Crj:CD(SD)IGS	Male	Oral	119	Goto (1997)
			Female	Oral	104	
IM-1-5	Rat	Crj:CD(SD)IGS	Male	Oral	141	Fujii (2002b)
			Female	Oral	132	
IM-1-2	Rat	Crj:CD(SD)	Male	Oral	> 5000	Mochizuki & Goto (1994a)
			Female	Oral		
IB-1-1	Rat	Crj:CD(SD)IGS	Male	Oral	> 2000	Kanaguchi (1999a)
			Female	Oral		

LD_{50}, median lethal dose

Table 82. Genotoxicity of other metabolites

Metabolite	End-point	Test object	Concentration	Result	References
IM-1-5	Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537	313, 625, 1250, 2500, 5000 µg/plate	Negative both with and without metabolic activation	Kanaguchi (1997)
IM-1-2	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 *Escherichia coli* WP2*uvrA*	313, 625, 1250, 2500, 5000 µg/plate	Negative both with and without metabolic activation	Mochizuki & Kanaguchi (1994c)
IB-1-1	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	313, 625, 1250, 2500, 5000 µg/plate	Negative both with and without metabolic activation	Kanaguchi (1999b)

hours after ingestion. The next day, his symptoms with regard to the effects of acetamiprid improved, and he was discharged from the hospital without complication. The blood concentration of acetamiprid on arrival, approximately 2 hours after ingestion, was 21.1 µg/ml (Todani et al., 2008).

Imamura et al. (2010) also described two cases of acute poisoning with an insecticide formulation containing acetamiprid for suicidal purposes. Both cases experienced severe nausea and vomiting, muscle weakness, hypothermia, convulsions and clinical manifestations, including tachycardia, hypotension, electrocardiogram changes, hypoxia and thirst in the case with the higher serum concentration of acetamiprid. The symptoms were partially similar to those of acute organophosphate intoxication. Supportive treatments for a variety of symptoms were sufficient for recovery, and both individuals were discharged without any complications 2 days after ingestion.

4. Literature review

A search of literature databases for published toxicological data on acetamiprid was performed. Only one study regarding the effect of acetamiprid on the immune system in female rats was found. A subacute toxicity study of acetamiprid was undertaken in 72 female Wistar rats in four groups (18 in each group). Three different concentrations of acetamiprid (25, 100 and 200 mg/kg bw) were administered orally to rats. Untreated rats served as controls. The antibody titre of sheep red blood cells in all the treated groups and a decrease in cell-mediated immune response were evaluated by delayed-type hypersensitivity reaction to dinitrofluorobenzene. The results revealed that in rats treated with 200 mg/kg bw, there were significant ($P \leq 0.01$) decreases in mean values of total leukocyte count and relative lymphocyte count. Globulin was also decreased significantly ($P \leq 0.01$) in acetamiprid-treated rats compared with controls. There was a significant decrease ($P \leq 0.01$) in spleen weight in rats treated

with the 200 mg/kg bw dose compared with controls. Histopathological examination of spleen revealed depletion of lymphocytes from Malpighian corpuscles in all treated groups in a dose-dependent manner. The results indicated that acetamiprid suppressed both cell-mediated immune response and antibody-forming ability of lymphocytes at dose levels above the maximum tolerated dose. Further, the substance used in the experiment was a 20% formulation of which the detailed identification is lacking. The study does not comply with guidelines and also suffers from a lack of reproducibility. For these reasons, this study was not taken into consideration in the current evaluation (Mondal et al., 2009).

Comments

Biochemical aspects

Acetamiprid is rapidly absorbed, with a maximum concentration in blood being achieved in approximately 2–3 hours. The extent of absorption was more than 90% of the administered radioactivity. Acetamiprid is widely distributed in the tissues, with highest concentrations being found in the adrenal gland, liver and kidney following oral administration to the rat. The concentration of radioactivity in the brain was lower than the concentration in blood at all time points. No sex differences were observed. The major route of elimination was via urine (53–65%). The recovery of the radioactivity excreted in the bile was less than 20% of the administered dose, which suggests that the bile is not a major route of excretion. The disappearance of radioactivity from the body of the rat was rapid, and there was no indication of accumulation in any tissue. Less than 1% of the administered radioactivity remained in the tissues by day 4 following dosing. The major radioactive compounds in the excreta of rats were acetamiprid (~5–7%), the demethylated compound IM-2-1 (~15–20%), the nicotinic acid derivative IC-O (~8–11%) and the IC-O glycine conjugate IC-O-Gly (~10%). In addition, MeS-IC-O, IM-1-4, IM-2-4, IM-O, IM-1-3 and IM-2-3 were detected, each at less than 2% of the dose. There were several unknown compounds in the urine, with a maximum abundance of 1%. The main metabolic pathway of acetamiprid in rats is the transformation to IM-2-1 by demethylation. IM-2-1 is further metabolized to IC-O, with the release of IS-1-1 and IS-2-1 after cleavage from the side-chains of NI-25 (parent compound) and IM-2-1.

Toxicological data

In mice and rats, the oral LD_{50} was in the range of 140–417 mg/kg bw. Dose-related reversible toxic signs (crouching, tremor, convulsion and mydriasis) were observed. The dermal LD_{50} in rats was greater than 2000 mg/kg bw. When acetamiprid was administered by inhalation through nose-only exposure, the LC_{50} was greater than 1.15 mg/l of air. Mydriasis in many rats and tremor and convulsion in a few rats were observed when acetamiprid was administered through inhalation (whole-body exposure), and these effects disappeared after 1 day. Acetamiprid was not an irritant in studies of ocular or dermal irritation in rabbits or a dermal sensitizer in the Magnusson and Kligman maximization test in guinea-pigs.

Short-term studies of oral toxicity in mice, rats and dogs were conducted using acetamiprid. These studies are characterized by similar toxic responses, such as decreased feed consumption and body weight.

In a 13-week study in mice, the NOAEL was 400 ppm (equal to 53.2 mg/kg bw per day), on the basis of a significant decrease in total cholesterol level in females at 800 ppm (equal to 106.1 mg/kg bw per day). Tremor, decreased body weight gain, decreased feed consumption, decreased haemoglobin concentration, decreased serum total cholesterol and glucose levels, decreased urinary pH, increased liver to body weight ratio and centrilobular hypertrophy were observed at higher doses.

In a 13-week study of oral toxicity in rats, the NOAEL was 200 ppm (equal to 12.4 mg/kg bw per day), on the basis of decreased body weight gain, decreased feed consumption and increased serum total cholesterol levels at 800 ppm (equal to 50.8 mg/kg bw per day).

In three oral dog studies (4 weeks, 90 days and 1 year), initial body weight losses and decreased body weight gains were observed in males and females receiving the highest dietary concentrations of acetamiprid. In the 4-week study, the NOAEL was 22 mg/kg bw per day. However, an overall NOAEL for the other two oral dog studies was 800 ppm (equal to 32 mg/kg bw per day).

In an 18-month study of toxicity and carcinogenicity in mice, decreased feed consumption was observed in males and females at 1200 ppm. At 400 ppm in males, body weights were decreased, and the body weight gain was statistically significantly decreased compared with controls through 13 weeks of study. At the end of 18 months, mean relative liver weights were increased in males and females receiving 1200 ppm and also in females receiving 400 ppm. On microscopic examination, treatment-related hepatocellular hypertrophy was seen in male and female mice receiving 1200 ppm after 12 and 18 months of treatment. These microscopic findings are considered to be an adaptive response of the liver to exposure to acetamiprid. The NOAEL was 130 ppm (equal to 20.3 mg/kg bw per day), based on transient decreased body weight observed at 400 ppm (equal to 65.6 mg/kg bw per day) in males. There was no evidence of any carcinogenic effect in mice.

A 2-year study of toxicity and carcinogenicity in rats demonstrated an increased incidence of clinical signs, such as rales, hunched posture and laboured breathing, in the 400 and 1000 ppm dose groups. The body weights of the 1000 ppm males and females and the 400 ppm females (until week 100) were statistically significantly lower than those of controls during the study. Trace to mild centrilobular hepatocellular hypertrophy and vacuolation were seen at 400 ppm and above. Incidences of mammary gland adenocarcinomas and hyperplasias were increased in females at 1000 ppm (equal to 60 mg/kg bw per day); however, incidence levels were within normal limits for ageing Crl:CD rats, and therefore these lesions are considered to be unlikely to be due to an endocrine or carcinogen effect of acetamiprid. Because the observation of rales at 160 ppm was not correlated to the other clinical signs, such as laboured breathing, moribundity, hunched posture and decreased activity, the NOAEL in this study was 160 ppm (equal to 7.1 mg/kg bw per day), based on hepatocyte vacuolation at 400 ppm (equal to 17.5 mg/kg bw per day). Acetamiprid was not carcinogenic in rats.

Acetamiprid was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. No significant result is obtained in these tests, except for chromosomal aberration induction in vitro. In vivo, there was no confirmation of chromosomal aberration in a number of tests, and there was no evidence of induction of DNA damage.

The Meeting concluded that acetamiprid is unlikely to be genotoxic in vivo.

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

In a two-generation study in rats, the NOAEL for systemic parental toxicity was 100 ppm (equal to 6.67 mg/kg bw per day), on the basis of a decline in body weights and feed consumption and an increased incidence of hepatocellular hypertrophy and vacuolation at 280 ppm (equal to 18.9 mg/kg bw per day) and above. The NOAEL for offspring toxicity was 280 ppm (equal to 13.9 mg/kg bw per day), on the basis of decreases in body weight gain in both generations and reduced postnatal survival in the F_2 offspring at 800 ppm (equal to 38.7 mg/kg bw per day). However, there are no effects on reproduction with treatment up to 800 ppm (equal to 38.7 mg/kg bw per day), the highest dose tested.

In a study of developmental toxicity in rats, the NOAEL for maternal toxicity was 16 mg/kg bw per day, based on decreased feed consumption and body weight gain during the treatment period in maternal rats in the 50 mg/kg bw per day group at scheduled sacrifice. The developmental NOAEL in rats was 16 mg/kg bw per day, based on the increased incidence of fetuses with shortening of the 13th rib at 50 mg/kg bw per day.

In a study of developmental toxicity in rabbits, the NOAEL for maternal toxicity was 15 mg/kg bw per day, based on decreased feed consumption and body weight gain during the treatment period at 30 mg/kg bw per day. The developmental NOAEL was 30 mg/kg bw per day, the highest dose tested.

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

In an acute oral neurotoxicity study, increased urination frequency and reduced locomotor activity were observed at doses of 30 mg/kg bw and above. Other clinical signs of neurotoxicity (e.g. hunching, tremors) were observed at higher doses. No apparent effects on sensory systems or evidence of neuropathology was seen. The NOAEL was 10 mg/kg bw, based on evidence of increased urination frequency (males) and a statistically significant reduction of locomotor activity (males) at 30 mg/kg bw.

A 13-week dietary neurotoxicity study in rats did not result in any changes that were considered indicative of neurotoxicity. The NOAEL was 200 ppm (equal to 14.8 mg/kg bw per day), on the basis of lower body weights and feed consumption at 800 ppm (equal to 59.7 mg/kg bw per day).

A developmental neurotoxicity study in rats revealed the NOAEL for maternal toxicity, developmental toxicity and developmental neurotoxicity to be 10 mg/kg bw per day, based on a reduction in body weight gain in dams during the first 3 days of dosing (gestation days 6–9), decreased feed consumption in F_0 animals, early postnatal mortality, reduced post-weaning body weights and deficits in auditory startle response without neuropathology or changes in brain morphometry in F_1 animals at 45 mg/kg bw per day.

Acetamiprid did not cause delayed neuropathy in hens.

Studies for immunotoxicity in mice (highest dose tested was 157 mg/kg bw per day) and rats (highest dose tested was 67.7 mg/kg bw per day) indicated no specific effect on immune function as assessed by the measurement of antigen-specific T cell–dependent antibody formation.

Toxicological data on impurities and metabolites

Acute toxicity studies and studies of genotoxicity have been undertaken for four compounds that are present as impurities in technical acetamiprid. None of them were genotoxic in a number of assays, and they had acute oral LD_{50} values in rats between 603 and greater than 5000 mg/kg bw. Nine compounds identified as plant metabolites are IM-1-3, IM-1-4, IM-2-1, IM-2-3, IM-2-4, IM-O, IC-O, IS-1-1 and IS-2-1. None were genotoxic in a number of assays, and they had acute oral LD_{50} values in rats between 900 and greater than 5000 mg/kg bw. The NOAEL following repeated exposure of rats to diets containing IM-1-4 for 13 weeks was 600 ppm (equal to 36.5 mg/kg bw per day), based on effects on spleen (increased pigments in splenic sinusoids) at 1800 ppm (112.2 mg/kg bw per day) in treated males. The NOAEL for IM-O in a 13-week study in rats was 800 ppm (equal to 48.9 mg/kg bw per day), on the basis of eosinophilic intranuclear inclusions seen in proximal tubular epithelium of kidneys at 4000 ppm (250.1 mg/kg bw per day) in males. All the impurities and metabolites were of lesser toxicity than the parent (acetamiprid).

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

Three cases of intentional poisoning with acetamiprid formulation have been reported. In one case, the concentration of acetamiprid in blood was measured at the time of reporting for treatment. In all cases, some signs similar to those associated with acute organophosphate intoxication were reported. Supportive treatments for a variety of signs were sufficient for recovery, and all recovered within 24–48 hours of the initiation of treatment.

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

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) of 0–0.07 mg/kg bw on the basis of the NOAEL of 7.1 mg/kg bw per day from the 2-year study of toxicity and carcinogenicity in rats, based on clinical signs and hepatocyte vacuolation seen at 17.5 mg/kg bw per day. A safety factor of 100 was applied. This ADI was supported by the NOAEL of 6.67 mg/kg bw per day observed in a

two-generation study of reproductive toxicity in rats on the basis of decreased parental body weight gain and feed consumption and hepatocyte vacuolation at 18.9 mg/kg bw per day.

The Meeting established an acute reference dose (ARfD) of 0.1 mg/kg bw on the basis of a NOAEL of 10 mg/kg bw in an acute neurotoxicity study in rats, based on evidence of neurotoxicity, decreased locomotor activity and increased urination frequency. This ARfD was supported by the NOAEL for maternal toxicity in the developmental neurotoxicity study of 10 mg/kg bw per day, based on reduced body weight gain in dams during the first 3 days of dosing (gestation days 6–9).

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen-month study of toxicity and carcinogenicity[a]	Toxicity	130 ppm, equal to 20.3 mg/kg bw per day	400 ppm, equal to 65.6 mg/kg bw per day
		Carcinogenicity	1200 ppm, equal to 214.6 mg/kg bw per day[b]	—
Rat	Two-year study of toxicity and carcinogenicity[a]	Toxicity	160 ppm, equal to 7.1 mg/kg bw per day	400 ppm, equal to 17.5 mg/kg bw per day
		Carcinogenicity	1000 ppm, equal to 60 mg/kg bw per day[b]	—
	Two-generation study of reproductive toxicity[a]	Offspring toxicity	280 ppm, equal to 13.9 mg/kg bw per day	800 ppm, equal to 38.7 mg/kg bw per day
		Reproductive toxicity	800 ppm, equal to 38.7 mg/kg bw per day[b]	—
		Parental toxicity	100 ppm, equal to 6.67 mg/kg bw per day	280 ppm, equal to 18.9 mg/kg bw per day
	Developmental toxicity study[c]	Maternal toxicity	16 mg/kg bw per day	50 mg/kg bw per day
		Embryo and fetal toxicity	16 mg/kg bw per day	50 mg/kg bw per day
	Acute neurotoxicity study[c]	Acute neurotoxicity	10 mg/kg bw	30 mg/kg bw
	Developmental neurotoxicity study[c]	Developmental neurotoxicity	10 mg/kg bw per day	45 mg/kg bw per day
Rabbit	Developmental toxicity study[c]	Maternal toxicity	15 mg/kg bw per day	30 mg/kg bw per day
		Embryo and fetal toxicity	30 mg/kg bw per day[b]	—
Dog	Ninety-day and 1-year studies of toxicity[a,d]	Toxicity	800 ppm, equal to 32 mg/kg bw per day	1500 ppm, equal to 55 mg/kg bw per day

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

Estimate of acceptable daily intake for humans

0–0.07 mg/kg bw

Estimate of acute reference dose

0.1 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 acetamiprid

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid and almost completely absorbed (> 90%)
Distribution	Widely distributed; highest concentrations in adrenal, liver and kidney
Potential for accumulation	No evidence of accumulation
Rate and extent of excretion	Rapid, more than 90% within 96 h, mainly via urine
Metabolism in animals	Moderately metabolized; the major radioactive compounds in the excreta of rats were acetamiprid itself and IC-O glycine conjugate
Toxicologically significant compounds (animals, plants and the environment)	Acetamiprid (parent compound)
Acute toxicity	
Rat, LD_{50}, oral	140–417 mg/kg bw
Rat, LD_{50}, dermal	> 2000 mg/kg bw
Rat, LC_{50}, inhalation	> 0.30 mg/l (whole-body exposure) > 1.15 mg/l (nose-only exposure)
Rabbit, dermal irritation	Non-irritant
Rabbit, ocular irritation	Non-irritant
Guinea-pig, dermal sensitization (Magnusson and Kligman test)	Non-sensitizer
Short-term studies of toxicity	
Target/critical effect	Increased cholesterol, decreased body weight, decreased feed consumption
Lowest relevant oral NOAEL	53.2 mg/kg bw per day (13-week study in mice)
Genotoxicity	
	Not genotoxic in vivo
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Increased clinical signs; hepatic vacuolation
Lowest relevant NOAEL	7.1 mg/kg bw per day (rats)
Carcinogenicity	Not carcinogenic in rats or mice
Reproductive toxicity	
Reproduction target/critical effect	None
Lowest relevant reproductive NOAEL	38.7 mg/kg bw per day, highest dose tested
Developmental target/critical effect	Skeletal anomalies
Lowest relevant developmental NOAEL	16 mg/kg bw per day (rat)
Neurotoxicity/delayed neurotoxicity	
Acute neurotoxicity target/critical effect	Motor activity and increased frequency of urination
Lowest relevant acute neurotoxic NOAEL	10 mg/kg bw
Subchronic neurotoxicity target/critical effect	Not neurotoxic (rats)
Developmental neurotoxicity target/critical effect	Deficits in auditory startle response
Lowest relevant developmental neurotoxic NOAEL	10 mg/kg bw per day (rat)

Immunotoxicity	
28-day immunotoxicity	Not immunotoxic (mice and rats)
Medical data	
	No significant health effects were reported among manufacturing personnel; however, three cases of intentional poisoning have been reported with some signs similar to those of acute organophosphate poisoning

Summary

	Value	Study	Safety factor
ADI	0–0.07 mg/kg bw	Two-year rat study (supported by parental toxicity in the multigeneration rat reproduction study)	100
ARfD	0.1 mg/kg bw	Acute neurotoxicity, rat (supported by maternal toxicity in the developmental neurotoxicity rat study)	100

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DICHLORVOS (addendum)

First draft prepared by
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Explanation

Dichlorvos is the International Organization for Standardization (ISO)–approved common name for 2,2-dichlorovinyl dimethyl phosphate (International Union of Pure and Applied Chemistry) or 2,2-dichloroethenyl dimethyl phosphate (Chemical Abstracts Service No. 62-73-7). It is a broad-spectrum organophosphorus insecticide, and, like other organophosphorus compounds, its mode of

action is via the inhibition of cholinesterase (ChE) activity. The toxicity of dichlorvos was evaluated by the Joint FAO/WHO Meeting on Pesticide Residues in 1965, 1966, 1967, 1970, 1977 and 1993. An acceptable daily intake (ADI) of 0–0.004 mg/kg body weight (bw) was established by the 1966 Meeting and maintained by all subsequent Meetings. Dichlorvos was reviewed by the present Meeting within the periodic review programme of the Codex Committee on Pesticide Residues.

All pivotal studies contained certificates of compliance with principles of good laboratory practice or good clinical practice and the Declaration of Helsinki, as appropriate.

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

[vinyl-1-^{14}C]Dichlorvos (703 kBq/mg) in olive oil was administered by gavage to one male and one female CF-1 mouse (0.2 mg/animal) and two male Syrian hamsters (0.56 mg/animal). A single female hamster was similarly dosed with 0.22 mg [vinyl-1-^{14}C]dichlorvos because 0.56 mg had proven fatal. One male human ingested 5 mg [vinyl-1-^{14}C]dichlorvos in 100 ml orange juice. Excreta (urine, faeces and carbon dioxide) were collected at various times for the analysis of radioactivity.

In mice, approximately 90% of the administered radioactivity was recovered in 24 hours, about 30% in each of the urine, expired carbon dioxide and carcass and 3% in faeces. In hamsters, total recovery of radioactivity was 88–98%, with most excreted within 24 hours of dosing. Approximately 50% of total recoverable radioactivity was excreted in expired carbon dioxide, 20% in urine and about 5% in faeces. Approximately 15% of radioactivity remained in the carcass 4 days after dosing, with about 2% in the gastrointestinal tract and 5% in skin; the presence of radioactivity in the carcass at this time may be due to the incorporation of ^{14}C into protein. Based on the levels of radioactivity in carbon dioxide, urine and the carcass, the gastrointestinal absorption was estimated to be greater than 90% in mice and hamsters. In the male human, approximately 27% was eliminated in carbon dioxide in 8 hours, compared with 9% in urine in 48 hours (~8% in 24 hours). Overall, the data indicated similar excretion patterns in mice, hamsters and humans (Hutson & Hoadley, 1972).

In the pilot phase of the study by Cheng (1989), [vinyl-1-^{14}C]dichlorvos (radiochemical purity 100%) was administered to Crl:CD(SD)BR rats (two of each sex) as a single gavage dose of 1.2 mg/kg bw in deionized water. Excreta were collected at 0–12 and 12–24 hours, then daily for 7 days. There were no mortalities or clinical signs. Total recovery of radioactivity was approximately 95%, with radioactivity detected in expired carbon dioxide (36%), the carcass (26%), urine (16%), faeces (13%), the tubing used to connect the carbon dioxide trap system (3%), cage wash (0.2%) and the charcoal filter used to trap any volatiles (0.06%). There was no difference in the level of radioactivity in excreta between males and females.

In the main phase of the study, five rats of each sex per group were administered [vinyl-1-^{14}C]-dichlorvos as a single intravenous dose of 1 mg/kg bw or as a single gavage dose of 0.8 or 21 mg/kg bw or unlabelled dichlorvos in 15 daily gavage doses of 0.8 mg/kg bw followed by a single radiolabelled dose of 0.8 mg/kg bw on the 16th day. The control group consisted of two untreated rats. Excreta were collected at 0–6, 6–12 and 12–24 hours post-dosing, then daily for 7 days. Survivors were sacrificed after 7 days, and tissues were collected for radiochemical analysis. One female from the 21 mg/kg bw group died at 2.5 hours post-dosing, whereas all other rats in this group displayed cholinergic signs (tremors and salivation); no details of the duration of these signs were given. Dark urine was noted on a number of occasions (typically ≥ 72 hours post-dosing) in three males and three females given the single intravenous dose of 1 mg/kg bw and in three males from the 0.8 mg/kg bw repeated-dose group; the toxicological significance of this observation is unclear.

Following all doses and routes, there was almost complete recovery of radioactivity (89–98%), with the maximum level detected in carbon dioxide (40–58%), followed by the carcass (13–26%), urine (10–17%) and faeces (4–7%). The liver contained 3.5–4.8% of the administered radioactive dose, with all remaining tissues containing 1–1.5%. Based on the levels of radioactivity in carbon dioxide, the carcass, urine, liver and other tissues following oral dosing (0.8 or 21 mg/kg bw), gastrointestinal absorption was estimated to be 92–95%. There was no difference in the proportion of radioactivity in excreta following oral or intravenous dosing; similarly, there did not appear to be any difference between the low and high oral doses or the repeated oral doses. The majority (~90%) of radioactivity was excreted by 24 hours, although low levels were still detectable at the last sampling interval. In carbon dioxide and urine, the highest concentrations of radioactivity occurred at 0–6 hours, with the levels reasonably consistent across all dosing regimens. Radioactivity then steadily declined over the remainder of the collection period, with low levels (~1%) still detectable at 144–168 hours. In faeces, two peaks of radioactivity were detected in all groups: the major one at 12–24 hours and a second smaller one at 144–168 hours.

Following all dosing regimens, the highest tissue radioactivity was detected in the carcass (13–26%) and liver (3.5–4.8%), which were the only tissues to have radioactivity levels higher than those in blood (0.32–0.5%). The next highest level of radioactivity was in the kidneys (0.23–0.45%), with the lowest radioactivity level detected in fat. There was no apparent difference in the tissue distribution of radioactivity following intravenous or oral dosing and between single or multiple oral doses (Cheng, 1989).

[vinyl-1-^{14}C]Dichlorvos (purity > 95%; 751 MBq/mmol) in water was applied to the shaved backs of 12 male Crl:CD(SD)BR rats at 3.6, 36 or 360 µg/animal (equal to 0.5, 3 and 30 µg/cm^2, respectively) under a non-occlusive dressing. Urine, faeces and carbon dioxide were collected at various times until sacrifice. At 10 hours, all rats were anaesthetized, the dressing was removed and the application site was washed. Blood and urine were sampled from four rats per group, with the rats then sacrificed and bladder contents collected and pooled with the final urine sample. The skin at the application site was excised, and the remaining carcass was kept for analysis. Cage rinses were also collected for analysis. New protective dressings were affixed to the remaining rats, and these procedures were repeated at 24 and 102 hours (360 µg group) or 120 hours (3.6 and 36 µg groups).

Recovery of radioactivity was approximately 90% across all doses. A substantial proportion of radioactivity evaporated from the skin surface, as shown by the relatively large level of radioactivity (38–56%) detected in the charcoal filter attached to the dressing. The next highest level of radioactivity was detected in the skin at the application site (12–20%), the first skin wash (7–13%), the dressing at 10 hours post-application (6–12%) and the carcass (2–6%). The level of radioactivity in exhaled carbon dioxide, urine, faeces and blood ranged from 2% to 5%, from 1% to 2%, from 0.1% to 1.6% and from 0.05% to 0.16%, respectively; this indicated that the main excretion pathway of absorbed radioactivity was via carbon dioxide. The total level of dermal absorption (calculated as the sum of the percentage of radioactivity in the carcass, skin, urine, faeces, blood and carbon dioxide) was approximately 22–30% across all doses, with the majority occurring within 10 hours of application. The level of dermal penetration (calculated as the sum of the per cent recovery in the carcass, urine, faeces and carbon dioxide) was also consistent across all doses and times and was approximately 6–11% (Jeffcoat, 1990).

1.2 Biotransformation

Metabolites of dichlorvos were analysed in urine collected from mice, hamsters and a human dosed orally with [vinyl-1-^{14}C]dichlorvos (see above study by Hutson & Hoadley, 1972) by paper chromatography and isotope dilution analysis. Results of this analysis are summarized in Table 1.

Table 1. Interspecies comparison of urinary dichlorvos metabolites

Metabolite	% of administered radioactivity		
	Mice	Hamsters	Human
Hippuric acid	0.6	1	0.4
Desmethyl dichlorvos	19	Not measured	0.2
Urea	0.6	Not measured	0.1

From Hutson & Hoadley (1972)

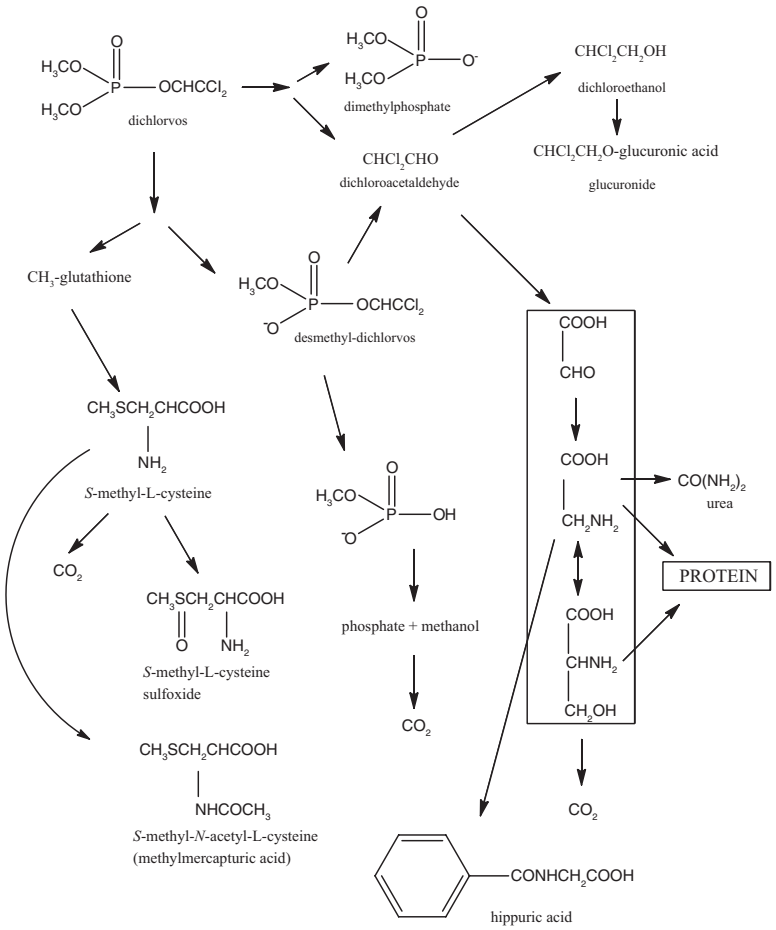
In the supplementary study by Cheng (1991), metabolites were analysed in pooled (0–24 hours) urine and faecal samples obtained in the main phase of the study by Cheng (1989). In faeces, 52–78% of radioactivity was extractable, and 23–57% was non-extractable; the identity of the non-extractable radioactivity was not specified. The concentration of radioactivity was approximately 5–8 times higher in urine than in faeces. The only metabolites to be definitively identified in excreta were hippuric acid and urea. In faeces, the levels of hippuric acid and urea were less than 6% and 3–30% of total faecal radioactivity, respectively, whereas in urine, they comprised 4–24% and 19–33% of total urinary radioactivity, respectively. These findings indicate that the metabolites contributing the majority of radioactivity present in excreta were unidentified. Treatment of urine samples with β-glucuronidase increased the level of radioactivity in the organic fraction by 6–13%, indicating that a proportion of urinary metabolites were glucuronidated. However, the identity of these glucuronidated metabolites was not determined.

Changes to the thin-layer chromatogram following storage (7 days for urine samples and 24 hours for faecal samples) and poor recoveries suggested that dichlorvos metabolites were volatile or were degraded to volatile compounds. Mass spectrometric analysis confirmed the formation of urea and hippuric acid, but was unable to identify other metabolites. The author suggested that dichlorvos metabolites were dehalogenated due to the lack of a characteristic chlorine cluster in the mass spectra. Based on the relatively large amount of radioactivity excreted via expired carbon dioxide and the detection of radiolabelled urea and hippuric acid in excreta, the author proposed that the metabolism of dichlorvos involves a one-carbon pool biosynthesis pathway (Cheng, 1991).

An overview of the proposed metabolic pathways of dichlorvos in rats, as described by the previous Meeting (Annex 1, reference *70*), is given in Figure 1. The metabolism of dichlorvos involves two pathways. The first pathway involves the oxidative *O*-demethylation of dichlorvos to produce desmethyl-dichlorvos by a glutathione-dependent enzymatic system. Hydrolysis of the *O*-demethylated metabolite yields methylphosphate, phosphoric acid and methanol. The second (predominant) pathway involves the ester hydrolysis of the oxygen–vinyl bond of dichlorvos to generate dimethylphosphate and dichloroacetaldehyde. The latter is further metabolized to dichloroethanol or dichloroacetic acid, and then to dichloroethanol glucuronide, hippuric acid, urea and carbon dioxide.

Ageda et al. (2006) investigated the influence of temperature on the decomposition of dichlorvos (and 13 other organophosphorus insecticides) in fresh blood. Dichlorvos (unspecified purity) in methanol was added to fresh human blood at a final concentration of 10 µg/ml and incubated for 24 hours at 4 °C, room temperature or 37 °C. Dichlorvos was completely degraded within 1 hour at 37 °C, 2 hours at room temperature and 12 hours at 4 °C. Graphically presented data indicated that the half-life of dichlorvos was approximately 15 minutes at 37 ºC and less than 1 hour at room temperature.

Figure 1. Proposed metabolic pathways of dichlorvos in the rat



2. Toxicological studies

2.1 Acute toxicity

The results of studies of the acute toxicity of dichlorvos administered to different animal species are summarized in Table 2, with further details following.

(a) Oral administration

Rats

Technical dichlorvos (unspecified purity) was administered to groups of five fasted rats (RAC strain) of each sex as a single gavage dose of approximately 14, 43, 57, 71, 85, 99, 114 or 142 mg/kg bw in 0.5% or 1% (volume per volume [v/v]) polyethylene glycol. Rats were observed for a period of 7 days. Deaths occurred at and above 57 mg/kg bw in females and 85 mg/kg bw in males within an hour of dosing. At 114 and 142 mg/kg bw, all rats died within an hour of dosing, with the exception of one high-dose female that survived to day 7. Clinical signs were evident at every dose and were observed immediately after dosing; these included clonic–tonic spasms, cramps of the cheek muscles and tachypnoea. The severity of these signs increased with dose, with exophthalmos, prostration, lacrimation, secretion from the Harderian glands and dyspnoea also observed at higher doses. Survivors recovered within 5 days. Macroscopic abnormalities in decedents included congested livers and gastrointestinal tract bloating. No macroscopic abnormalities were observed in survivors. The median lethal dose (LD_{50}) was 108 mg/kg bw in males and 80 mg/kg bw in females; the combined LD_{50} was 92 mg/kg bw (Hurni & Sachsse, 1969a).

Table 2. Results of studies of acute toxicity of dichlorvos

Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	LC_{50} (mg/l)	Reference
Rat	RAC	Males and females	Oral	108 (males) 80 (females) 92 (males and females)	—	Hurni & Sachsse (1969a)
Rat	Wistar-derived albino	Males and females	Oral	57 (sample 1) 64 (sample 2)	—	Spanjers & Til (1979a,b)
Rabbit	Yellow Silver	Males and females	Oral	74	—	Hurni & Sachsse (1970)
Rat	RAC	Males and females	Dermal	210	—	Hurni & Sachsse (1969b)
Mouse	CF-1	Males and females	Inhalation (4 h, head only)	—	> 0.22	MacDonald (1982)
Rat	Wistar	Males and females	Inhalation (4 h, head only)	—	> 0.20	MacDonald (1982)
Rat	Wistar	Males and females	Inhalation (4 h, head only)	—	0.23	Debets (1986)

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

A sample of technical dichlorvos (unspecified purity) in 1% (v/v) polyethylene glycol was administered by gavage to groups of five male and female Wistar-derived albino rats at a dose of approximately 40, 47, 57, 68, 82 or 98 mg/kg bw. A second sample in 0.5% (v/v) polyethylene glycol was administered similarly at a dose of 68, 82, 98, 118 or 142 mg/kg bw. Clinical signs (sluggishness, ataxia and coma) occurred at and above 47 mg/kg bw (sample 1) or 82 mg/kg bw (sample 2); females were more sensitive than males. It was stated that survivors gradually recovered. There were no macroscopic abnormalities observed at necropsy. The LD_{50} values were 57 mg/kg bw for sample 1 and 64 mg/kg bw for sample 2 (Spanjers & Til, 1979a,b).

Rabbits

Technical dichlorvos (unspecified purity) was administered to groups of two fasted rabbits (Yellow Silver strain) of each sex as a single gavage dose of 46, 60, 78 or 100 mg/kg bw in polyethylene glycol (concentration unspecified). Rabbits were observed for 14 days, sacrificed and necropsied. Deaths occurred at every dose (1/4, 2/4, 2/4 and 4/4 at 46, 60, 78 and 100 mg/kg bw, respectively). Clinical signs were observed approximately 10–20 minutes after dosing and included dyspnoea, salivation, asynchronism of the extremities, clonic–tonic muscle spasms and, at later times, exophthalmos and lateral positioning. Survivors recovered within 24 hours. At necropsy, decedents had congested lungs and liver and haemorrhage in the thymus and stomach. No macroscopic abnormalities were observed in survivors. The LD_{50} was 74 mg/kg bw (Hurni & Sachsse, 1970).

(b) Dermal application

Rats

Technical dichlorvos (unspecified purity) in 10% (v/v) polyethylene glycol was applied to a skin area of approximately 10 cm^2 on the shaved backs of rats (RAC strain; three of each sex per group) at a dose of 114, 142, 213 or 284 mg/kg bw. The test material was applied under an occlusive

dressing for 24 hours. The application site was then washed, and the rats were observed for 7 days. There were no deaths or clinical signs at 114 or 142 mg/kg bw. At 213 mg/kg bw, three rats had died by day 7, whereas at 284 mg/kg bw, all rats had died by day 7. At these doses, clinical signs (clonic–tonic spasms of the limbs, prostration, exophthalmos, dyspnoea, lacrimation and secretion from the Harderian glands) occurred at 15–30 minutes after application. In survivors, signs had resolved by day 6. No skin irritation was observed. In decedents, congestion of the liver, spleen and kidney, bloating of the intestines and inflammation of the peritoneum were observed. In survivors, enlarged livers and bloating were recorded at necropsy. The LD_{50} was 210 mg/kg bw (Hurni & Sachsse, 1969b).

(c) Exposure by inhalation

Mice

Groups of five CF-1 mice of each sex were exposed (head only) to dichlorvos (purity > 97.8%) in particulate and vapour form for 4 hours at a nominal concentration of 0.22 mg/l, then observed for 14 days. A control group was exposed to compressed air under the same conditions. There were no deaths. Clinical signs included tremors (two males, five females), lethargy (all), paresis in the hind legs (one male, two females) and splayed gait (all), which resolved within 2 days. In survivors, body weight gains were comparable with those of the controls. There were no macroscopic abnormalities. The median lethal concentration (LC_{50}) (4 hours) for males and females was greater than 0.22 mg/l (MacDonald, 1982).

Rats

Groups of five Wistar rats of each sex were exposed (head only) to dichlorvos (purity > 97.8%) in particulate and vapour form for 4 hours at nominal concentrations ranging from 0.085 to 0.25 mg/l. Exposures were performed at different times (i.e. not concurrently). At the highest nominal concentration of 0.25 mg/l, three males died; however, analysis of the atmosphere indicated that it was oversaturated and contained dichlorvos in both particulate and vapour forms. Exposure of a new group of rats at a near-saturated atmosphere of 0.21 mg/l resulted in 100% mortality. There were no deaths at 0.14 mg/l, whereas a single male died at 0.085 mg/l. Further groups of rats were exposed to vapour concentrations of 0.206 and 0.198 mg/l, with no mortalities. Clinical signs were observed at every concentration and included piloerection, tremors, lethargy, ataxia, hypersensitivity to noise, hypothermia, hindlimb paresis and splayed gait. These signs had generally resolved within 3 days. In survivors, body weight gains were comparable with those of the controls. In the rats that died at the two highest concentrations (0.21 and 0.25 mg/l), pulmonary congestion and turgidity were observed at necropsy. The LC_{50} (4 hours) for males and females was greater than 0.20 mg/l (MacDonald, 1982).

Groups of five Wistar rats of each sex were exposed (head only) to aerosols of dichlorvos (purity > 97%) in acetone for 4 hours at an analytical concentration of 0.17, 0.20 or 0.24 mg/l. Rats were then observed for 14 days. No concurrent control group was included, although historical control data were provided in the study report. The average particle size was 2.3 µm (mass median aerodynamic diameter [MMAD]), with a mean geometric standard deviation of 3.1 µm. Deaths occurred from about 15 minutes to 2.5 hours after the commencement of exposure (combined totals of 1/10, 2/10 and 6/10 at 0.17, 0.20 and 0.24 mg/l, respectively). Clinical signs occurred at each exposure concentration and included lethargy, ataxia, tremors, hypopnoea and blood encrustation of the nose and eye, which resolved within 3–4 days. Minimal body weight gain occurred from day 0 to day 7 at 0.20 and 0.24 mg/l but recovered thereafter. Necropsy revealed haemorrhages of the lungs in 1/10, 0/10 and 3/10 rats at 0.17, 0.20 and 0.24 mg/l, respectively. Two rats exposed to dichlorvos at 0.24 mg/l had bloody trachea contents. The combined LC_{50} (4 hours) was 0.23 mg/l (Debets, 1986).

(d) Dermal and ocular irritation

Dichlorvos (purity > 97%) was administered undiluted (0.2 ml) via a 3 cm^2 gauze patch to the left flank skin of three female New Zealand White rabbits for 70 or 200 minutes under a semi-occlusive dressing. A gauze patch without dichlorvos was attached to the right flank skin as the control. One rabbit died 3 hours after sample application. The remaining rabbits developed overt signs of toxicity (tremors and ataxia) at this same time; consequently, exposure was terminated 20 minutes later. These rabbits had recovered by the next day. Slight oedema and slight to well-defined erythema were observed 24 hours after removal of the gauze patch. In both rabbits, the erythema had resolved by 14 days, whereas oedema had resolved by either 48 hours or 14 days. As a result of the high acute dermal toxicity, it was not possible to classify the skin irritancy of the test substance (Mulder, 1986a).

Following instillation of 0.1 ml dichlorvos (purity 97%) into the eye of one female New Zealand White rabbit, death occurred within 7 minutes, which was preceded by lethargy, convulsions, muscle contraction and immobility. As a consequence, no further rabbits were dosed (Mulder, 1986b).

(e) Skin sensitization

In a maximization test, nine female Dunkin-Hartley guinea-pigs were induced intradermally and topically with 5% and 25% dichlorvos, respectively. Animals were challenged with 0.005%, 0.05% or 0.5% dichlorvos, and reactions were scored at 24 and 48 hours. Positive responses at 0.005%, 0.05% and 0.5% were scored in 0%, 56% and 100% of animals, respectively, at 24 hours and in 0%, 22% and 67% of animals, respectively, at 48 hours. There was a dose-related increase in the grade of the reaction. In a separate test, animals induced with triforine exhibited a positive response when challenged with dichlorvos. This published, non-guideline study indicated that dichlorvos was a skin sensitizer (Ueda et al., 1994).

2.2 Short-term studies of toxicity

Mice

In a 1980 study that was re-reported by Konishi, Mennear & Bernard (1989) because of deficiencies in the original English translation, B6C3F1 mice (9–12 of each sex per group) were exposed ad libitum to dichlorvos (purity 97.26%) in drinking-water at various concentrations for 10 weeks. In the first experiment, mice were exposed to dichlorvos at a concentration of 0, 25, 50, 100, 200 or 400 mg/l (target doses were 0, 3.75, 7.5, 15, 30 and 60 mg/kg bw per day, respectively). There were no treatment-related mortalities. The final body weight of animals in all treated groups was about 10% lower than that of the control group. Organ weights were unremarkable.

In a second experiment, mice were exposed to dichlorvos at a concentration of 800, 1600, 3200, 5000 or 10 000 mg/l (target doses were 120, 240, 480, 750 and 1500 mg/kg bw per day, respectively). This experiment lacked a concurrent control group. Deaths occurred at and above 1600 mg/l (all mice at 5000 and 10 000 mg/l; 7/11 males and 1/9 females at 3200 mg/l; and 1 male at 1600 mg/l). Body weight gain was reduced at and above 1600 mg/l. In decedents, there was macroscopic evidence of lung discoloration and intestinal haemorrhage. Histopathology revealed pulmonary haemorrhage and haemorrhage/necrosis of the small intestine. Pulmonary congestion was observed in survivors.

In a third experiment, no treatment-related effects were evident in mice exposed to dichlorvos concentrations of 400 or 800 mg/l (60 and 120 mg/kg bw per day, respectively).

Rats

In a 1978 range-finding study that was re-reported by Enomoto, Mennear & Bernard (1989) because of deficiencies in the original English translation, groups of 10 F344 rats of each sex were

exposed to dichlorvos (purity 97.3%) via the drinking-water for 4–5 hours/day for 6 weeks at a nominal dose of 0, 5, 10, 20, 40 or 80 mg/kg bw per day. At 80 mg/kg bw per day, two rats of each sex died (days 12–42), with one male and one female dying in the 5 and 40 mg/kg bw per day groups, respectively (days 27 and 24, respectively). Decreased spontaneous motor activity was observed at 80 mg/kg bw per day, whereas lacrimation was observed across all treated groups (incidence and duration unspecified). At 80 mg/kg bw per day, body weight gain was lower than that of the control group (~25% lower at termination); it was stated that feed and water consumption were also lower in this group. Also at 80 mg/kg bw per day, the absolute weights of a number of organs (e.g. lung, liver, heart) were more than 20% lower than those of the control group; however, as there was no difference in relative organ weights, these findings are attributable to the lower terminal body weights of animals in this group. There were no treatment-related gross pathological or histopathological abnormalities.

In a range-finding study, dichlorvos (unspecified purity) was administered by gavage to five female Crl:CD(SD)BR rats per group at 0, 0.1, 10 or 20/40 mg/kg bw per day for up to 7 days. At 40 mg/kg bw per day, one rat died on the first day of dosing, with the four remaining rats appearing hypoactive and displaying tremors. Consequently, this dose was reduced to 20 mg/kg bw per day following a 2-day washout period. Rats from the control and 0.1 mg/kg bw per day groups appeared normal. Plasma and erythrocyte ChE activities were significantly lower ($P < 0.05$) than in the control group at 10 and 20 mg/kg bw per day; the level of inhibition was approximately 73% for plasma ChE activity and 30% for erythrocyte ChE activity at both doses (Kleeman, 1988a).

Dichlorvos (purity 98.3%) in deionized water was administered by gavage to male and female Crl:CD(SD)BR albino rats (10 of each sex per group) for 5 days/week for 13 weeks at 0, 0.1, 1.5 or 15 mg/kg bw per day. Rats were observed for approximately 30 minutes after each dose and then twice daily. Body weight and feed consumption were recorded weekly. Ophthalmic examinations were performed pretreatment and at termination. Blood (fasted) was sampled during weeks 7 and 14 for the analysis of plasma and erythrocyte ChE activities. Rats were sacrificed at week 14 and necropsied. Blood was collected for the analysis of haematology and clinical chemistry parameters. Brain ChE activity was analysed. Histopathology was performed on tissues from the control and high-dose groups.

With the exception of a single control female, there were no deaths. Treatment-related clinical signs were confined to the high-dose group and included salivation (seven males and four females) and urine stains (seven males and five females), both occurring 30–60 minutes post-dosing during weeks 6–12. There were no treatment-related ophthalmic abnormalities and no effect on body weight gain or feed consumption. Dose-related inhibition of plasma, erythrocyte and brain ChE activities occurred, reaching statistical significance ($P < 0.05$) at and above 1.5 mg/kg bw per day for plasma and erythrocyte ChE activities and at 15 mg/kg bw per day for brain ChE activity (Table 3). The significantly lower erythrocyte ChE activity determined for females at 0.1 mg/kg bw per day during week 14 is not considered toxicologically significant because it was less than 20% of control activity and was not corroborated by similar statistical differences during week 7, in males or for plasma ChE activity.

Selected haematology and clinical chemistry parameters are summarized in Table 4. At 15 mg/kg bw per day in both sexes, red blood cells, haemoglobin and haematocrit were significantly lower than in the control group ($P < 0.05$). In males, haemoglobin and haematocrit were also significantly lower than in the control group at 1.5 mg/kg bw per day. Mean corpuscular volume was significantly elevated ($P < 0.05$) in females at 15 mg/kg bw per day, whereas cholesterol was significantly elevated ($P < 0.05$) in males at this same dose. The significant elevation in cholesterol in males at 15 mg/kg bw per day may have been due to a single outlying rat with high cholesterol (166 mg/dl). An examination of historical control data indicated that the majority of red blood cell parameters were within the range of normal

Table 3. Inhibition of cholinesterase activity in rats

ChE	Mean % inhibition relative to controls					
	0.1 mg/kg bw per day		1.5 mg/kg bw per day		15 mg/kg bw per day	
	Males	Females	Males	Females	Males	Females
Plasma						
- week 7	10	0	29*	15	65*	58*
- week 14	10	0	18	6	35*	47*
Erythrocyte						
- week 7	2	10	24*	25*	47*	42*
- week 14	8	8*	25*	24*	42*	34*
Brain						
- week 14	0	4	4	4	28	49*

From Kleeman (1988b)
* $P < 0.05$

Table 4. Clinical pathology findings in rats[a]

Parameter	0 mg/kg bw per day		0.1 mg/kg bw per day		1.5 mg/kg bw per day		15 mg/kg bw per day	
	Males	Females	Males	Females	Males	Females	Males	Females
Red blood cells (10^6/µl)	9.68	8.86	9.85	8.76	8.95	8.55	8.78*	7.69*
Haemoglobin (g/dl)	16.2	15.8	16.2	15.4	14.6*	15.4	14.8*	13.8*
Haematocrit (%)	53.2	52.1	53.8	51.4	49.0*	50.9	49.4*	46.9*
Mean corpuscular volume (fl)	55	59	55	59	55	60	56	61*
Cholesterol (mg/dl)	75	91	78	89	80	92	102*	103

From Kleeman (1988b)
* $P < 0.05$ compared with the concurrent control
[a] Results expressed as the mean.

biological variation. The only parameters that were marginally outside of this range were slightly lower haemoglobin and higher mean corpuscular volume in high-dose females and slightly higher haematocrit in the control and low-dose male groups. Therefore, the statistically significant changes in red blood cell parameters are within the bounds of normal biological variation and on this basis are not considered toxicologically significant. The study authors concluded that these findings were not biologically significant. Absolute and relative organ weights were unremarkable. There were no treatment-related macroscopic or histopathological abnormalities.

The no-observed-adverse-effect level (NOAEL) was 1.5 mg/kg bw per day, based on the inhibition of brain ChE activity and salivation at the highest dose of 15 mg/kg bw per day (Kleeman, 1988b).

Dogs

Dichlorvos (100% purity) was administered orally to four Beagle dogs of each sex per group in gelatine capsules at 0, 0.05 (0.1 for the first 3 weeks of the study), 1 or 3 mg/kg bw per day for 52 weeks. Observations for mortalities and clinical signs were made daily, with cage-side observations

Table 5. Occurrence of emesis in dogs

Dog	Number of weeks when emesis was observed							
	0 mg/kg bw per day		0.05 mg/kg bw per day		1 mg/kg bw per day		3 mg/kg bw per day	
	Males	Females	Males	Females	Males	Females	Males	Females
1	0	0	1	4	1	2	4	3
2	1	0	0	0	2	2	2	1
3	0	1	0	2	4	1	29	9
4	0	0	0	0	3	0	3	0

From Markiewicz (1990)

made for about 1 hour after dosing. Physical examinations were performed weekly. Body weights and feed consumption were recorded 1 day prior to the commencement of dosing, weekly from weeks 1 to 16 and then every 4 weeks until termination. Ophthalmic examinations were performed pretreatment and at termination. Blood and urine were sampled pretreatment and during weeks 26 and 52 for haematology, clinical chemistry and urinalysis. Plasma and erythrocyte ChE activities were measured in samples collected from non-fasted dogs pretreatment during weeks 2, 6, 13, 26, 39 and 52. After 52 weeks, survivors were sacrificed and necropsied. ChE activity was measured in the cerebellum. Organs were weighed, and tissues were examined histopathologically.

There were no deaths. One high-dose male may have been overdosed (unconfirmed) during week 33 and exhibited ataxia, salivation and dyspnoea. There was a dose-related increase in emesis, which was more pronounced in males than in females (Table 5). With the exception of one high-dose male, emesis tended to predominate in treated dogs during the second half of the study.

There was no statistically significant difference in mean body weight or body weight gain between treated and control groups. In high-dose males, mean body weight over the first 7 weeks of treatment was lower than mean pretreatment body weight and remained lower than the control body weight until week 20, when there was no apparent difference. An examination of individual animal data indicated that a single high-dose male lost about 15% of its pretreatment body weight over the first 3 weeks of dosing and never completely recovered. This animal also exhibited the highest incidence of emesis over the dosing period (29 of the 38 events). There was no treatment-related effect on feed consumption.

There were no treatment-related ophthalmic abnormalities or effect on any haematology, clinical chemistry or urinalysis parameter.

Dose-related inhibition of plasma, erythrocyte and brain ChE activities occurred, reaching statistical significance at 1 and 3 mg/kg bw per day (Table 6). At 0.05 mg/kg bw per day, transient inhibition of plasma and erythrocyte ChE activities occurred during weeks 2 and 6, respectively. This group received 0.1 mg/kg bw per day for 3 weeks, which was then decreased to 0.05 mg/kg bw per day from week 4 because inhibition of plasma ChE activity occurred after 12 doses. At the lower dose, no inhibition of plasma ChE activity occurred at subsequent sampling points. The study authors attributed the inhibition of erythrocyte ChE activity during week 6 in the low-dose group (24% in males and 50% in females) to the residual effect on erythrocytes from the higher dose of 0.1 mg/kg bw per day, despite the dogs having received the lower dose of 0.05 mg/kg bw per day for 4 weeks. The lack of plasma ChE inhibition during week 6 supports this conclusion.

There were no treatment-related macroscopic abnormalities, changes in organ weight or histological abnormalities. The NOAEL was 0.05 mg/kg bw per day based on occurrence of emesis and the inhibition of brain ChE activity at and above 1 mg/kg bw per day (Markiewicz, 1990).

Table 6. Inhibition of cholinesterase activity in dogs

ChE	Mean % inhibition relative to the mean pretreatment activity							
	0 mg/kg bw per day		0.05 mg/kg bw per day		1 mg/kg bw per day		3 mg/kg bw per day	
	Males	Females	Males	Females	Males	Females	Males	Females
Plasma								
- week 2	7	0	21	26	59	59	67	65
- week 6	12	2	11	10	59	57	74	74
- week 13	0	0	0	0	48*	41*	68*	61*
- week 26	0	0	0	0	39*	51*	71*	74*
- week 39	7	0	8	6	48*	44*	65*	68*
- week 52	2	1	12	0	53*	52*	72*	65*
Erythrocyte								
- week 2	0	0	2	0	34	33	75	68
- week 6	0	0	24	50	65	63	94	90
- week 13	0	1	7	7	54*	52*	87*	82*
- week 26	0	0	3	3	43*	38*	85*	83*
- week 39	3	8	8	13	44*	40*	81*	79*
- week 52	0	0	0	2	53*	45*	85*	81*
Brain (cerebellum)								
- week 53	0	0	12	0	23*	7	47*	29*

From Markiewicz (1990)
* $P < 0.05$

2.3 Long-term studies of toxicity and carcinogenicity

No new long-term studies of toxicity and carcinogenicity were submitted for evaluation. Previous Meetings evaluated a total of 11 carcinogenicity studies conducted in mice and rats that were dosed orally (diet, drinking-water or gavage) or by inhalation; previous summaries of these studies are reproduced below. In addition, two drinking-water studies conducted in mice and rats, which had been considered previously, were re-evaluated because the study reports had been re-written and the data statistically reanalysed by the authors.

Mice

Groups of 100 male and 100 female C57BL/6/Bln mice (5–6 weeks old) received by gavage 0.2 mg dichlorvos (purity 97% in 0.2 ml water) per mouse, freshly prepared, 2 or 3 times per week for 50 weeks. Control groups received by gavage either 0.2 ml water 3 times per week (about 50 males and 50 females) or no treatment (35 males and 35 females). Surviving animals were sacrificed after 110 weeks. This strain of mice is known for the spontaneous occurrence of mixed lymphomas (reticulum cell sarcoma type B).

From the age of 12 months onwards, some animals in all groups developed interstitial pneumonia. The incidence of mixed lymphomas was decreased in both test groups (26–60% in control groups, 23–30% in treated groups). An increased incidence of focal hyperplasia (transitional cell hyperplasia) of the urinary bladder was found in both dichlorvos groups (0–8% in control groups, 5–10% in treated groups). The authors concluded that no neoplastic lesions were found that could be attributed to the treatment of the animals with dichlorvos (Annex 1, reference *70*).

Dichlorvos was not co-carcinogenic in C57BL/6/Bln mice when administered by gavage 3 times per week at 0.2 mg per animal to mice subcutaneously injected with 50 µg *N*-nitrosodiethylamine

per animal weekly for 50 weeks followed by an observation period of up to 110 weeks (Annex 1, reference *70*).

Two groups of 50 male and 50 female B6C3F1 mice were fed 1000 or 2000 parts per million (ppm) dichlorvos (purity > 94%) in corn oil in the diet for 2 weeks. Owing to severe signs of intoxication, doses were lowered to 300 and 600 ppm for the following 78 weeks. Samples of the diets analysed during the study showed that time-weighted average concentrations were 318 and 635 ppm. Matched controls consisted of 10 mice of each sex; the pooled controls from simultaneous studies with other compounds consisted of 100 male and 80 female mice. All surviving mice were killed at 92–94 weeks. Animals were observed twice daily for clinical signs.

Alopecia and rough hair coats were noted in many treated animals, particularly in the male groups, beginning at week 20 and persisting throughout the study. The average body weights of the high-dose mice of both sexes were slightly decreased compared with controls. The low-dose female group showed 74% survival at 90 weeks compared with 84% and 90% in high-dose and control groups, respectively. There was no significant increase in the incidence of tumours attributable to dichlorvos in either sex.

Two squamous cell carcinomas of the oesophagus (one in a low-dose male and one in a high-dose female), one papilloma of the oesophagus in a high-dose female and three cases of focal hyperplasia of the oesophageal epithelium in low-dose males were recorded in the treated mice. The significance of the findings in the treated mice was considered uncertain because of insufficient information concerning the spontaneous incidence of these lesions and lack of statistical significance within the experiment. Dichlorvos (up to 635 ppm in the diet, equivalent to 95 mg/kg bw per day) was not demonstrated to be carcinogenic in this study (Annex 1, reference *70*).

Studies in B6C3F1 mice given dichlorvos in drinking-water at concentrations of 0, 400 or 800 mg/l for 2 years and in CFE rats exposed to dichlorvos by inhalation at 0, 0.05, 0.48 or 4.7 mg/m^3 for 23 hours/day for 2 years were summarized in IPCS (1989). There was no evidence of carcinogenic effects in these studies (Annex 1, reference *70*).

The above drinking-water study in B6C3F1 mice was re-reported and statistically reanalysed by Konishi, Mennear & Bernard (1989) as a result of deficiencies in the original English translation. B6C3F1 mice (50 of each sex per group) were exposed ad libitum to dichlorvos in the drinking-water at a dose of 58 or 95 mg/kg bw per day in males and 56 or 102 mg/kg bw per day in females. Mice were observed for clinical signs daily. Body weight and water consumption were recorded weekly during the 1st year of exposure and every 2 weeks thereafter. Feed consumption was not recorded. Following sacrifice or death, all mice were necropsied, organ weights were recorded and tissues were examined histopathologically. Survival was not affected adversely by treatment, and it was stated that there were no clinical signs; statistical analysis revealed a significant increase in survival in high-dose males ($P = 0.039$). There was a treatment-related reduction in water consumption (44% and 60% lower than for controls in males and 25% and 35% lower than for controls in females at the low and high doses, respectively), which suggests reduced palatability. Graphically presented data indicated that mean body weights were lower than those of the controls at both doses over the course of the study; the final body weight was significantly lower than that of the control group ($P < 0.01$ or 0.05).

Mean absolute liver, spleen, brain, heart, lung and gonad weights of high-dose males were significantly lower than those of the controls, whereas mean absolute kidney and pancreas weights of high-dose females were significantly lower than those of the controls (*P* values unspecified); these reductions probably reflect the reduced terminal body weight. Mean relative liver, lung and spleen weights in high-dose males were lower than those of the controls, but not significantly so (liver: 18%

and 31% lower than the control weights at the low and high doses, respectively; spleen: 43% lower than the control weights at the high dose; lung: 20% and 32% lower than the control weights at the low and high doses, respectively). In the absence of any histopathology of these organs, none of these findings are considered treatment related.

Testicular atrophy was observed in 0 of 49, 5 of 47 and 4 of 47 males at 0, 58 and 95 mg/kg bw per day, respectively, but there were no statistically significant differences between the treated and control groups. Squamous cell hyperplasia of the glandular stomach occurred in 0 of 49, 4 of 47 and 3 of 47 males at 0, 58 and 95 mg/kg bw per day, respectively, and in 1 of 43 high-dose females.

Squamous cell papillomas of the glandular stomach were observed in 1 of 47 low-dose and 1 of 47 high-dose males, and squamous cell carcinoma was observed in 1 of 47 low-dose males. None of these findings were statistically significant compared with the control group, in which no such lesions were observed. These findings were not replicated in females, for which squamous cell papilloma of the glandular stomach was observed in 2 of 41 control and 1 of 38 low-dose females. In males, the occurrence of thyoma was increased, but not significantly (0/49, 4/47 and 1/47 at 0, 58 and 95 mg/kg bw per day, respectively). In the absence of a dose–response relationship, this finding was not considered treatment related. In males, malignant fibrous histiocytoma was increased, but again not significantly (1/49, 4/47 and 3/47 at 0, 58 and 95 mg/kg bw per day, respectively). The lack of a dose–response relationship and corroborative observations in females suggests that these findings are unrelated to treatment.

Dichlorvos was not carcinogenic in mice at doses up to 95 and 102 mg/kg bw per day in males and females, respectively. The occurrence of squamous cell hyperplasia with the apparent progression to papillomas suggests treatment-related proliferative changes in the glandular region of the stomach (Konishi, Mennear & Bernard, 1989).

Groups of 50 B6C3F1 mice were given dichlorvos (purity 99%) by corn oil gavage at doses of 0, 10 or 20 (males) or 0, 20 or 40 (females) mg/kg bw per day, 5 days/week, for 103 weeks. The dose volume of the corn oil was 10 ml/kg bw. Body weights were recorded once weekly for the first 12 weeks and then monthly. Animals were observed twice per day for clinical signs. Necropsy and histological examinations were performed on all moribund animals or at the end of the study.

Body weight gain and survival did not significantly differ between treated and control groups. No compound-related clinical signs were observed. Blood ChE activity was not determined during the study.

The incidences of forestomach squamous cell papillomas were 1 out of 50, 1 out of 50 and 5 out of 50 in control, low-dose and high-dose males, respectively, and 5 out of 49, 6 out of 49 and 18 out of 50 in control, low-dose and high-dose females, respectively. The positive trend was statistically significant in both sexes, whereas by pairwise comparison, only the incidence in high-dose females was significantly higher than that in controls. Two forestomach squamous cell carcinomas were seen in high-dose females, and none in the other groups. No increase in the incidence of forestomach hyperplasia was seen in the dosed mice compared with vehicle controls (10–20%). In female mice, the incidence of adenomas and adenomas or carcinomas (combined) of the pituitary gland (12/45, 6/45 and 6/44 in control, low-dose and high-dose groups, respectively) and the incidence of lymphomas (16/50, 11/50 and 9/50 in control, low-dose and high-dose groups, respectively) showed a significant negative trend. Based on the increased incidence of forestomach papillomas, the NOAEL was 10 mg/kg bw per day (Annex 1, reference *70*).

Rats

Groups of 40 male and 40 female weanling CD rats were fed diets containing dichlorvos (purity 93%) at a nominal concentration of 0, 0.1, 1, 10, 100 or 500 ppm for 2 years. Diets were prepared weekly. Five males and five females from each group were killed after 6, 12 or 18 months. Analysis

of diet samples showed a considerable loss of dichlorvos associated with a gradual increase in dichloroacetaldehyde content (average concentrations ranging from 0.01 to 28.6 ppm). The average actual concentrations of dichlorvos in each diet were 0, 0.05, 0.5, 4.7, 47 and 230 ppm. No cholinergic signs were observed. No effects were seen on behaviour, mortality rate, weight gain, feed consumption, terminal body and organ weights, haematology or urinalysis. Plasma and erythrocyte ChE activities were measured 13 times during the study. In the 100 ppm group, plasma and erythrocyte ChE activities were reduced to 60–90% and 50–90% of control activities, respectively; in the 500 ppm group, the activities were reduced to 20–70% and 20–60% of control activities, respectively. Activities were higher towards the end of the study. Brain ChE activity was decreased in the highest dose group by 45–47%, 24–43%, 24–38% and 5–15% after 6, 12, 18 and 24 months, respectively. Histological examination of major organs (liver, heart, lungs, kidneys, spleen, brain, gonads, pituitary, adrenals and thyroids) revealed hepatocellular fatty vacuolization in all 500 ppm rats and in most females and several males at 100 ppm. No effect was seen on serum total proteins or albumin:globulin ratio or on hexobarbital sleeping time. The tumour incidence was comparable with that of the control group. The NOAEL, based on brain ChE inhibition, was 100 ppm (actual concentration 47 ppm, equivalent to 2.4 mg/kg bw per day) (Annex 1, reference *70*).

Groups of 50 male and 50 female weanling CFE rats were exposed (whole body) to nominal air concentrations of 0, 0.05, 0.5 or 5 mg dichlorvos (purity 97%) per cubic metre for 23 hours/day for 2 years. The average actual dichlorvos concentrations were 0, 0.05, 0.48 and 4.7 mg/m^3. Body weight gain was reduced in the two highest dose groups. After 2 years of exposure, plasma and erythrocyte ChE activities were reduced by 20–30% at 0.5 mg/m^3 and by more than 60% in the highest-dose group; brain ChE activity was reduced by 10% (statistically significant) at 0.5 mg/m^3 and by 80% in the highest-dose group. No effects attributable to dichlorvos were seen on appearance, feed consumption, haematological or blood chemistry values, organ weights, or gross or microscopic examinations of major organs. Ultrastructural examinations of bronchi and alveoli of rats exposed to 0 or 5 mg/m^3 showed no differences between the two groups. It was concluded that 2-year exposure of CFE rats to dichlorvos at 0.05 mg/m^3 did not cause observable adverse effects. It should be noted that in this study, the rats were exposed not only by inhalation but also via their feed and drinking-water and by grooming. This resulted in additional oral ingestion of dichlorvos (Annex 1, reference *70*).

Osborne-Mendel rats (50 of each sex) were fed 1000 ppm dichlorvos (purity > 94%) in corn oil for 3 weeks. As a result of severe cholinergic signs, the dose was reduced to 300 ppm for the remaining 77 weeks. Another group (50 males and 50 females) was fed 150 ppm dichlorvos for 80 weeks. Samples of the diets analysed during the study showed that time-weighted average concentrations were 330 and 150 ppm, respectively. Matched controls consisted of 10 rats of each sex; the pooled controls from simultaneous studies of other compounds consisted of 60 rats of each sex. Animals were observed twice daily for clinical signs. All surviving rats were killed after 110–111 weeks.

The average body weights of the high-dose rats of both sexes were slightly decreased compared with controls. There was no significant increase in the incidence or type of tumours attributable to dichlorvos in either sex. Dichlorvos (up to 330 ppm in the diet, equivalent to about 30 mg/kg bw per day) was not demonstrated to be carcinogenic in this study (Annex 1, reference *70*).

Groups of 70 and 100 rats of each sex received 0.1 mg dichlorvos (purity 97%) per rat in 0.2 ml water by gavage 2 or 3 times per week, respectively, for 60 weeks. Thereafter, the animals were observed for another 51 weeks. A control group of 60 animals of each sex received 0.2 ml water 3 times per week. From the age of 10 months onwards, the incidence of focal hyperplasia of urinary bladder and of renal pelvis increased in males but decreased in females from both test

groups compared with controls. No neoplastic lesions were found that could be attributed to treatment (Annex 1, reference *70*).

A study in which Fischer 344 rats were given drinking-water containing dichlorvos at concentrations of 0, 140 or 280 mg/l for 108 weeks was summarized by IPCS (1989). There was no evidence of carcinogenicity (Annex 1, reference *70*).

The preceding study was re-reported and statistically reanalysed by Enomoto, Mennear & Bernard (1989) owing to deficiencies in the original English translation. F344 rats (50 or 51 of each sex per group) were exposed to dichlorvos (purity 97.3%) via the drinking-water for 4–5 hours/day for 104 weeks at doses of 8 and 18 mg/kg bw per day in males and 10 and 22 mg/kg bw per day in females. Survival was unaffected by treatment. Chromodacryorrhoea was observed in treated rats during the first 2 weeks of exposure, whereas high-dose females also exhibited tremors and were hypersensitive when touched (incidence and duration unspecified). The mean body weight of high-dose males was consistently lower (5–16%) than that of the control group throughout the study; the mean body weight of females was 0–13% lower than that of the control group. At the low dose, mean body weight was somewhat lower than that of the controls (2–6% in males, 2–10% in females). Feed and water consumption were unaffected by treatment. Body weight and feed consumption data were not statistically analysed.

The mean liver weight of high-dose males was approximately 30% lower than that of the controls (no statistical difference); in the absence of any pathology, this decrease is attributable to the lower terminal body weight of this group (there was no difference in relative liver weight). All other organ weights were unremarkable. In males, ulceration of the glandular stomach was marginally increased in the two treated groups (2%, 10% and 6% at 0, 8 and 18 mg/kg bw per day, respectively), but not significantly so; in the absence of a dose–response relationship or a concomitant increase in ulceration/irritation or proliferative changes in other parts of the upper digestive tract (e.g. the oesophagus or forestomach), this finding is not considered treatment related. In males, the incidence of mononuclear cell leukaemia was higher in the two treated groups compared with the controls (6%, 15% and 13% at 0, 8 and 18 mg/kg bw per day, respectively), but there was no dose–response relationship, and pairwise comparisons detected no significant differences. When the incidence of mononuclear cell leukaemia and lymphocytic leukaemia was combined (6%, 19% and 17% at 0, 8 and 18 mg/kg bw per day, respectively), the incidence in the low-dose group but not in the high-dose group was significantly higher ($P = 0.048$) than that in the control group; there was no dose-related trend. On the basis of these observations, dichlorvos was not carcinogenic.

Groups of 50 male and 50 female F344/N rats were administered dichlorvos (purity 99%) by corn oil gavage at 0, 4 or 8 mg/kg bw per day, 5 days/week, for 103 weeks. Body weights were recorded once weekly for the first 12 weeks and then monthly. Animals were observed twice daily for clinical signs. Necropsy and histological examinations were performed on all moribund animals or at the end of the study.

Mild diarrhoea was observed in treated animals. ChE activities were not determined during the study. No significant differences in mean body weights or survival were observed between any groups of either sex. An increased incidence of cytoplasmic vacuolization of liver was observed in dosed males, and an increased incidence of cortical cytoplasmic vacuolization of adrenal glands was observed in all dosed males and in low-dose females. The incidence of pancreatic adenomas (cross and horizontal tissue sections were analysed) in control, low-dose and high-dose groups was 25 out of 50, 30 out of 50 and 33 out of 50 in males and 2 out of 50, 3 out of 50 and 6 out of 50 in females, respectively. The increased incidence in treated males was statistically significant. The incidence of

mononuclear cell leukaemia (11/50, 20/50 and 21/50 in control, low-dose and high-dose groups, respectively) was significantly increased in the treated male rats compared with controls. The incidence of mammary gland adenomas or fibroadenomas in female rats was 11 out of 50 in controls, 19 out of 50 in the 4 mg/kg bw per day group and 17 out of 50 in the 8 mg/kg bw per day group. The incidence in the low-dose group was only slightly higher than the historical control values. Two mammary gland carcinomas were observed in each of the control and low-dose groups. The authors concluded that there was evidence that dichlorvos is carcinogenic to rats. The 1993 Meeting observed that the incidence of pancreatic adenomas in male control rats was unusually high, and therefore the higher incidence found in treated animals was considered to be of questionable biological significance. The increased incidence of mononuclear cell leukaemia, which is usually high and variable in this strain of rats, was also of questionable biological significance (Annex 1, reference *70*).

Dichlorvos at 8 or 16 mg/kg bw per day was administered by gavage to groups of 8–12 male F344 rats, either with or without leukaemia transplant, for 5 days/week. Other groups of rats were either not treated or given the leukaemia transplant only. At 70 days post-transplant, the animals were killed. The rats dosed with dichlorvos developed the disease earlier, and the rate of tumour progression was increased. Three out of 16 transplant recipients dosed with 16 mg/kg bw per day died of leukaemia during the last week of dosing. The severity of the mononuclear cell leukaemia in the transplant recipients, as measured by histopathological examination of spleen and liver, was correlated with the changes in tumour growth rates. However, no dose–response relationship was found for spleen weight and white blood cell count (Annex 1, reference *70*).

2.4 Genotoxicity

The 1993 Meeting evaluated numerous in vitro and in vivo genotoxicity studies on dichlorvos, which indicated that the compound is genotoxic in bacteria and cultured mammalian cells, but was not clastogenic in vivo except under conditions where an unusually high tissue dose can be attained. It was concluded that dichlorvos and its major metabolite, dichloroacetaldehyde, had been adequately tested for genotoxicity. The 1993 Meeting noted that dichlorvos methylates deoxyribonucleic acid (DNA) in vitro at a rate that is 8–9 orders of magnitude lower than the rate of phosphorylation. Therefore, DNA alkylation is not likely to occur at doses of dichlorvos that are not inhibitory to erythrocyte or brain ChE.

The results of supplementary genotoxicity studies on dichlorvos are summarized in Table 7. All unpublished studies contained statements of compliance with principles of good laboratory practice or international/national test guidelines. Consistent with previous observations, dichlorvos tested positive for a number of in vitro genotoxicity end-points at relatively high concentrations but was generally negative for genotoxicity in vivo.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

Dichlorvos (purity 98.3%) was administered to two parental generations of outbred Crl:CD(SD) BR rats (30 of each sex per dose) and their offspring via the drinking-water at concentrations of 0, 5, 20 or 80 mg/l. Doses received during the pre-mating periods were 0, 0.5, 2 and 7 mg/kg bw per day for males and 0, 0.7, 2.5 and 9.5 mg/kg bw per day for females. The doses received by females were 0, 0.6, 2 and 8 mg/kg bw per day during gestation and 0, 1, 4.5 and 17 mg/kg bw per day during

Table 7. Results of genotoxicity assays

End-point	Test object	Concentration	Purity (%)	Results	Reference
In vitro studies					
Gene mutation[a]	Mouse lymphoma L5178Y cells	±S9, 0.013–1.0 µl/ml (Trial 1), 0.0067–0.5 µl/ml (Trial 2), DMSO vehicle	97.5	Positive (±S9)	Ford, Killeen & Baxter (1986)[b]
Gene mutation	Mouse lymphoma L5178Y cells	−S9, 0–200 nl/ml (Trial 1), 0–50 nl/ml (Trial 2), ethanol vehicle	NR	Positive	Myhr et al. (1990)
Gene mutation	CHO cells (*hgprt* locus)	−S9, 0–150 µg/ml, +S9, 0–800 µg/ml, DMSO vehicle	NR	Positive (±S9)	Oshiro et al. (1991)
Chromosomal aberrations	CHO cells	−S9, 0–160 µg/ml (Trials 1 & 2), +S9, 0–1600 µg/ml (Trial 1) and 0–1000 µg/ml (Trial 2), DMSO vehicle	NR	Positive (±S9)	Anderson et al. (1990)
Micronucleus	CHO cells	−S9, 0–150 µg/ml, +S9, 0–800 µg/ml, DMSO vehicle	NR	Positive (±S9)	Oshiro et al. (1991)
Micronucleus	Human lymphoblastoid cells (AHH-1)	−S9, 0–50 ng/ml, DMSO vehicle	NR	Positive	Mattiuzzo et al. (2006)
Sister chromatid exchange[c]	CHO cells	−S9, 0–16 µg/ml (Trial 1) and 0–10 µg/ml (Trial 2), +S9, 0–500 µg/ml (Trials 1 & 2), DMSO vehicle	NR	Positive (±S9)	Anderson et al. (1990)
DNA damage (alkaline unwinding assay)[d]	Rat hepatocytes	−S9, 0–2000 µmol/l, vehicle unspecified	99.0	Positive	Yamano (1996)
In vivo studies					
Mutation[e]	λlacZ transgenic mouse (multiple tissues)	4.4 or 11 mg/kg bw (ip), 5 × 11 mg/kg bw (ip), DMSO or PBS vehicle		Negative (single dose) Positive (repeated dose)	Pletsa et al. (1999)
Dominant lethal	CD-1 male mice	0, 1, 3 or 10 mg/kg bw per day for 5 days in corn oil (ip)	98.4	Negative	Ford, Killeen & Ignatoski (1985a)[b]
Dominant lethal	CD-1 male mice	0, 8, 16 or 32 mg/kg bw per day for 5 days in corn oil (ip)	97.5	Negative	Ford & Killeen (1987)[b]
Chromosomal aberrations	Wistar rats (bone marrow)	0.97, 1.29 or 1.94 mg/kg bw, 5 days/week, for 6 weeks in water (gavage)	98	Negative	Nehéz, Tóth & Dési (1994)
Chromosomal aberrations	ICR male mice (bone marrow and spermatocytes)	0, 12.5 or 25 mg/kg bw per day for 5 days in water (gavage)	98.09	Negative	Putman & Shadly (1992)[b]
Sister chromatid exchange	B6C3F1 mice (bone marrow)	0, 3, 10 or 30 mg/kg bw in corn oil (ip)	NR	Negative	Putman (1985)[b]

Table 7 (continued)

End-point	Test object	Concentration	Purity (%)	Results	Reference
Micronucleus	CD-1 mice (bone marrow)	0, 4, 13 or 40 mg/kg bw for 2 days in corn oil (ip)	98.4	Negative	Ford, Killeen & Ignatoski (1985b)[b]
Micronucleus	CD-1 mice (bone marrow)	3.1%, 6.3% and 12.5% of LD_{50} in DMSO (dermal)	NR	Negative	Schop, Hardy & Goldberg (1990)
Comet assay[f]	Male ICR mice	100 mg/kg bw in olive oil (po)	NR	Positive	Sasaki et al. (2000)

CHO, Chinese hamster ovary; DMSO, dimethyl sulfoxide; ip, intraperitoneal; LD_{50}, median lethal dose; NR, not reported; PBS, phosphate-buffered saline; po, per os; S9, 9000 × *g* rat liver supernatant

[a] −S9, 2.3- to 13.3-fold increase in mutation frequency; +S9, 2.0- to 3.7-fold increase in mutation frequency.

[b] Unpublished study.

[c] −S9, authors described response as "weakly positive".

[d] DNA single-strand breaks concomitant with lipid peroxidation and reduced cell viability; no modulation by cytochrome P450 status.

[e] No intergroup differences in methylated DNA adducts or λlacZ mutants in bone marrow, white blood cells, liver, spleen, lung, brain or sperm cells following a single dose; 3-fold increase ($P < 0.001$) in mutation frequency in the liver following 5 times weekly doses of dichlorvos.

[f] Study of limited regulatory value (no vehicle control or assessment of cell viability).

lactation, respectively. Following the 10-week dosing period, F_0 females were mated with one F_0 male for 21 days. If mating was unsuccessful during the 1st week, then the male was replaced with a different male from the same dose group for the remaining 14 days. F_0 males were sacrificed after completion of the mating period. From day 20 of gestation, females were observed twice daily for evidence of littering and allowed to rear the F_1 litters until weaning (day 21 postpartum). Four days after birth, the size of each litter was randomly adjusted to four pups of each sex. At least one male and one female F_1 weanling per litter were randomly chosen to produce the F_2 generation. These F_1 weanlings (30 of each sex per dose) were exposed to the same drinking-water concentrations of dichlorvos as their parents for at least 11 weeks and then mated when about 14–17 weeks of age. Ten F_1 weanlings of each sex per dose were also randomly assigned for necropsy. All remaining F_1 weanlings were macroscopically examined and discarded. F_{2a} litters were weaned at day 21 postpartum, with 10 weanlings of each sex per dose randomly assigned for necropsy. All remaining F_{2a} weanlings were macroscopically examined and discarded. Owing to low breeding performance, F_1 females were subjected to a 3-week pre-breeding vaginal cytology examination to examine estrous cycling and then remated with a separate group of untreated males. In addition, F_1 males were subjected to a more extensive assessment for reproductive toxicity.

Standard gross toxicological end-points (deaths, clinical signs, body weight and feed and water consumption) were measured throughout all phases of the study. At birth and throughout lactation, pups were counted, sexed and examined macroscopically. Standard reproduction indices were calculated. Plasma, erythrocyte and brain ChE activities were analysed in all F_0 and F_1 parental rats at termination (after the mating period for males and after lactation for females). All parental rats were necropsied, and reproductive tissues were examined histopathologically. Brain weights were also recorded.

Parental rats: There were no treatment-related deaths or clinical signs. In F_0 dams, mean body weight gain during gestation was 9–15% lower than for the controls at all doses, but only the difference at 20 mg/l was statistically significant ($P < 0.05$). Otherwise, body weight gains were comparable across all groups. Feed consumption was unaffected by treatment in F_0 parents, with an approximate 6–11% increase ($P < 0.05$) occurring in F_1 males at 80 mg/l. At 80 mg/l, significantly reduced ($P < 0.05$) water consumption occurred in both parental generations of rats during the pre-mating (6–14% in males, 8–19% in females; $P < 0.05$), gestation (13–20%) and lactation (3–20%) periods. The

Table 8. Inhibition of cholinesterase activity in F_0 and F_1 parental rats

ChE	% inhibition relative to the concurrent negative control group					
	5 mg/l		20 mg/l		80 mg/l	
	Males	Females	Males	Females	Males	Females
Plasma						
- F_0	4	12	29*	55*	41*	83*
- F_1	15*	9	26*	54*	58*	81*
Erythrocyte						
- F_0	7	23*	29*	39*	57*	60*
- F_1	14*	17*	32*	42*	55*	58*
Brain						
- F_0	1	6	15*	26*	53*	59*
- F_1	1	2	6*	32*	40*	60*

From Tyl, Myers & Marr (1992, 1993)
* $P < 0.01$

toxicological significance of this finding is unclear, but it may be attributable to the reduced palatability of the drinking-water.

Results of ChE activity measurements in both parental generations are summarized in Table 8. Significant ($P < 0.05$), dose-related inhibition of plasma, erythrocyte and brain ChE activities occurred at and above 20 mg/l in both parental generations. At the lowest drinking-water concentration (5 mg/l), significant inhibition ($P < 0.05$) of erythrocyte ChE activity also occurred, but not at a level considered toxicologically significant.

There were no treatment-related macroscopic abnormalities in F_0 parental rats of either sex. There were no treatment-related microscopic abnormalities in F_0 parental females. Microscopic examination of F_0 parental males revealed seminiferous tubule degeneration and atrophy (graded as minimal) in treated groups, which tended to be observed only in one testis; these findings are summarized in Table 9, which also includes data from F_1 parental males for comparison. These findings were not considered treatment related because of the absence of a dose–response relationship, the lack of consistency between F_0 and F_1 rats and the consistency of these findings with the incidence recorded in controls from two other studies.

The mean terminal body weight of F_1 males from the 80 mg/l group was significantly lower ($P < 0.05$; ~10%) than that of the controls, which meant that the majority of relative organ weights were correspondingly higher. There were no treatment-related effects on any sperm parameters (epididymal sperm count, testicular spermatid count, abnormal sperm or the number of males with abnormal epididymal fluid). Gross necropsy and histopathology of males were unremarkable. The body weight and combined testes weight of the untreated males used in the remating of F_1 females were normal. In F_1 females examined prior to remating, there was a significant decrease ($P < 0.05$) in cycling at 80 mg/l (86%, 100%, 87% and 63% at 0, 5, 20 and 80 mg/l, respectively) and a significant increase ($P < 0.05$) in females with abnormal cycles (14%, 23%, 7% and 43% at 0, 5, 20 and 80 mg/l, respectively). The authors stated that the decreased or abnormal cycling was due to persistent or prolonged estrus. There was no treatment-related effect on cycle length.

Reproduction: Reproduction indices were unremarkable in F_0 parental rats and F_1 litters. Selected reproduction and lactation indices for F_1 parental rats/F_2 litters are summarized in Table 10. At 80 mg/l, male fertility and female fertility were approximately 20–25% lower than in the controls in both litters. The pregnancy index was lower at 80 mg/l than in the controls in both litters. The number of females with live litters was also reduced at 80 mg/l (both litters), with an apparent

Table 9. Incidence of seminiferous tubule degeneration and atrophy in male rats

Parameter	Incidence (absolute number of rats)			
	0 mg/l[a]	5 mg/l	20 mg/l	80 mg/l
F_0	*n* = 30	*n* = 30	*n* = 30	*n* = 30
Total number of affected rats	0	2	6	3
Unilateral degeneration	0	0	2	1
Bilateral degeneration	0	0	0	2
Unilateral atrophy	0	1	3	0
Bilateral atrophy	0	1	2	0
F_1	*n* = 30	*n* = 30	*n* = 30	*n* = 30
Total number of affected rats	1	4	3	1
Unilateral degeneration	1	3	1	1
Bilateral degeneration	0	0	0	0
Unilateral atrophy	0	2	2	0
Bilateral atrophy	0	0	0	0

From Tyl, Myers & Marr (1992, 1993)

[a] Control data from two separate studies indicated that unilateral and bilateral atrophy occurred in up to 3/30 and 1/30 rats, respectively, whereas unilateral and bilateral degeneration occurred in up to 2/30 and 1/30 rats, respectively.

Table 10. Reproduction and lactation indices for F_{2a} and F_{2b} litters

Index	0 mg/l	5 mg/l	20 mg/l	80 mg/l
F_{2a}				
Fertility index				
- female	71	74	76	55
- male	76	68	74	55
Pregnancy index	81	74	84	55
Females with live litters/group (%)	53	47	53	33
Stillbirth index	6.3 ± 5.9	1.4 ± 1.4	9.9 ± 5.6	10.7 ± 9.0
Seven-day survival index	100	86	87	81
Lactation index	100	84	87	80
F_{2b}				
Fertility index				
- female	76	70	63	50
- male	75	69	62	50
Pregnancy index	79	73	65	50
Females with live litters (%)	66	63	57	37
Stillbirth index	5.1 ± 3.5	9.0 ± 4.3	6.6 ± 3.2	19.1 ± 10.2
Seven-day survival index	99	87	93	89
Lactation index	99	85	92	86

From Tyl, Myers & Marr (1992, 1993)

increase in the stillbirth index in the F_{2b} litter. Marginal reductions in pup survival (day 7) and the lactation index were noted in both litters. None of these differences were statistically significant. All other reproduction and lactation indices were unremarkable.

Offspring: At 80 mg/l, the mean body weights of F_{1a} pups were approximately 8% lower than those of the control group from day 7 postpartum ($P < 0.05$ at days 14 and 21). A similar trend was evident in both F_{2a} and F_{2b} litters (5–15% lower body weight than the controls). There were no other treatment-related effects on offspring.

The NOAEL for parental toxicity was 0.5 mg/kg bw per day, based on the inhibition of brain ChE activity at and above 2 mg/kg bw per day. The NOAEL for offspring toxicity was 2 mg/kg bw per day, based on lower pup weights in the F_1 and F_2 generations at 8 mg/kg bw per day. The NOAEL for reproductive toxicity was 2 mg/kg bw per day, based on reduced fertility and pregnancy indices, increased stillbirths in the F_2 generation and abnormal cycling in F_1 maternal rats at 8 mg/kg bw per day (Tyl, Myers & Marr 1992, 1993).

In a published, non-guideline spermatotoxicity study, dichlorvos (purity 99%) in corn oil was administered by gavage to groups of 10 male Wistar rats at doses of 0, 5 or 10 mg/kg bw per day, 6 days/week, for 9 weeks. There were no deaths. Limb tremors were observed in an unspecified number of high-dose rats. The mean body weight of high-dose rats was approximately 6% lower ($P < 0.05$) than that of the control group. At the highest dose, mean absolute and relative heart weights were approximately 12% and 6% lower ($P < 0.05$), respectively, than those of the controls, whereas relative adrenal weight was about 20% higher ($P < 0.05$); no microscopic analysis of these tissues was undertaken. There was no treatment-related effect on the mean absolute or relative weight of the prostate, seminal vesicle, testis or epididymis. There was no treatment-related effect on plasma testosterone.

Results of the analysis of selected sperm parameters and ChE activity are presented in Table 11. There was no treatment-related effect on sperm count. Although sperm motility at 0 but not 60 minutes was significantly lower ($P < 0.05$) than that in the controls in both treated groups, the magnitude of the difference was small, there was no dose–response relationship and there were no effects on eight other motion parameters. On this basis, the difference is unlikely to be biologically relevant. There was no treatment-related effect on the incidence of abnormal sperm heads, whereas the incidences of broken sperm and cytoplasmic droplets were significantly higher ($P < 0.05$) than in the controls at the high dose. It was stated that other (unspecified) morphological parameters analysed were unaffected by treatment, including the conventional assessment of double-tailed sperm. Histopathology revealed a significant increase (no *P*-value) in cytoplasmic vacuolization in the epithelial cells of the epididymis at the highest dose (3.6 versus 1.7 in the control; units unspecified), but there were no other intergroup differences in microscopic findings, including any effects on the testes. There was a significant decrease ($P < 0.05$) in the ratio of adenosine triphosphate to adenosine diphosphate in sperm at the high dose at 60 minutes, which does not corroborate the slight reduction in motility reported at 0 minutes. Inhibition of erythrocyte and testicular acetylcholinesterase (AChE) activities occurred at both doses, but was statistically significant ($P < 0.05$) only at the high dose. Plasma butyryl ChE activity was significantly reduced ($P < 0.05$) at the high dose. There was a dose-related increase in the concentration of dimethyl phosphate detected in urine (106 and 197 mg/g creatinine at 5 and 10 mg/kg bw per day, respectively); neither diethylphosphate nor diethylthiophosphate was detected (Okamura et al., 2009).

In a non-guideline study, effects on the testis and epididymis were examined in groups of five male Wistar rats that were administered a gavage dose of dichlorvos (purity 76%) in water at 0 or 10 mg/kg bw per day for 48 days. Approximately 65% of the spermatozoa residing in the lumen of the cauda epididymis retained the cytoplasmic droplet, compared with 0% in the control group. In the treated group, the duration of motility of the spermatozoa released from the cauda epididymis was significantly lower ($P < 0.001$) than that of the controls (12.4 versus 28.3 minutes, respectively). The authors hypothesized that the reduced sperm motility was due to the retention of the cytoplasmic droplet. Although this study had a number of limitations (low purity of test material, the use of

Table 11. Sperm parameters and cholinesterase inhibition in male rats[a]

Parameter	0 mg/kg bw per day	5 mg/kg bw per day	10 mg/kg bw per day
Sperm count (×10^6/g cauda)	386	383	409
Sperm motility (% motile sperm)			
- at 0 min	77	63*	65*
- at 60 min	36	21	23
Sperm morphology (%)			
- abnormal sperm head	2	2	2
- broken sperm	1	3	5*
- cytoplasmic droplet	6	9	12*
ChE activity (% inhibition relative to control)			
- plasma	0	18	29*
- erythrocyte	0	40	44*
- testicular	0	22	25*

From Okamura et al. (2009)
* $P < 0.05$
[a] Results expressed as means.

a single dose level, small group sizes and limited end-points), it appears to confirm the reduction in sperm motility reported in the preceding study by Okamura et al. (2009) (Akbarsha, Latha & Murugaian, 2000).

Oral et al. (2006) reported effects on the endometrium (including apoptosis) in seven female Wistar rats that received dichlorvos (unspecified purity) in corn oil by gavage at 4 mg/kg bw per day, 5 days/week, for 4 weeks in the presence and absence of vitamins C and E (50 mg/kg bw intramuscularly and 20 mg/kg bw intraperitoneally, respectively).

Rats showed obvious signs of intoxication, including muscle fasciculations (described as severe), reduced body weight gain and serum AChE inhibition. It was stated that dichlorvos caused a significant ($P < 0.05$) decrease in the number of estrous cycles and duration of each phase compared with control rats. Although this observation appears consistent with the study of Tyl, Myers & Marr (1992, 1993; see above) at a dose of 8 mg/kg bw per day, the lack of the presentation of any data in the paper prevents such a comparison from being made. The endometrial effects were described as an irregular epithelial lining of the endometrium, disorganization of the glandular epithelium, shrinkage of epithelial cells in the endometrial glands and pyknotic nucleus in epithelial cells. However, as these findings were not quantified, graded or compared with appropriate historical control data, they were not interpretable as presented. Apoptosis was assessed as a significant ($P < 0.05$) increase in caspase-3 (but not caspase-9) expression in epithelial and stromal cells; the increase in a single marker of apoptosis is unlikely to be biologically relevant. This non-guideline study was considered to have limited regulatory value.

In a recently published, non-guideline study that was designed to examine testicular toxicity, dichlorvos (purity 98%) in corn oil was administered by gavage to groups of 18 male Wistar rats at a dose of 1.6 mg/kg bw per day for up to 7 weeks. An additional group of 18 rats was dosed with dichlorvos and vitamins C and E (200 mg/kg bw per day each by gavage).

There were no deaths. Clinical signs were unreported. It was stated that feed consumption was reduced in dichlorvos-treated rats, but no data were presented. In rats treated with dichlorvos

or with dichlorvos plus antioxidants and then sacrificed during week 4 or 7, mean body weight was significantly lower ($P < 0.05$) than that of the controls (~13% and ~23% lower than that of the controls during weeks 4 and 7, respectively). In fact, both dichlorvos groups lost approximately 6–13% of their pretreatment body weights. Absolute and relative testis weights were significantly lower ($P < 0.05$) than the control weights in dichlorvos-treated rats (in either the presence or absence of antioxidants) sacrificed at weeks 4 (~20% and ~30%, respectively) and 7 (~10% and ~20%, respectively). There were no intergroup differences in sperm count. Sperm motility was significantly reduced ($P < 0.05$) in dichlorvos-treated rats (in either the presence or absence of antioxidants) compared with the control group in rats sacrificed during week 7 only (~4% versus ~12% sperm motility in the control group). The mean incidence of abnormal epididymal sperm morphology was marginally although significantly higher ($P < 0.05$) in dichlorvos-treated rats compared with the controls in rats sacrificed during weeks 4 and 7 (~1.5% in the control versus ~2.5% in treated groups); the biological relevance of this increase is questionable, particularly in light of the absence of any effect on sperm count.

Graphically presented data illustrated small but statistically significant reductions ($P < 0.05$) in follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone levels in dichlorvos-treated rats sacrificed during weeks 4 and 7 (FSH: ~3 versus 4 mIU/ml in the control; LH: 1 versus 1.3 mIU/ml in the control; testosterone: ~2.5 versus 4.2 ng/ml in the control). The authors made no comparison of these results with age-matched historical control data, and therefore the biological relevance of these findings is unclear. It is worth noting that the study by Okamura et al. (2009), which was also conducted in Wistar rats, detected no effect on serum testosterone at doses of 5 and 10 mg/kg bw per day for 9 weeks. A number of histological and ultrastructural abnormalities were reported in the seminiferous tubules or testes of rats sacrificed after 4 or 7 weeks of dichlorvos treatment, but no attempt was made to quantify these findings or compare them with appropriate historical control data. On this basis, the toxicological relevance of these observations is unclear. This study is considered to have limited regulatory value (Dirican & Kalender, 2011).

Dichlorvos (unspecified purity) in ethanol was tested for interactions with the estrogen and androgen receptors in in vitro transactivation assays using MCF-7 and Chinese hamster ovary (CHO) cells, respectively. The effects on MCF-7 cell proliferation and cytochrome P450 (CYP) 19 aromatase activity in human placental microsomes were also analysed. Cytotoxicity occurred in MCF-7 and CHO cells at dichlorvos concentrations greater than 50 µmol/l and greater than 100 µmol/l, respectively. Dichlorvos had no effect on cell proliferation or estrogen receptor transactivation in MCF-7 cells or on microsomal aromatase activity. The study authors classified dichlorvos as a "very weak" androgen receptor antagonist based on the approximately 20% inhibition of R118 (a synthetic androgen) at 0.1 nmol/l by dichlorvos at 20 µmol/l (Andersen et al., 2002).

(b) Developmental toxicity

Rats

Dichlorvos (purity 96.86%; water vehicle) was administered by gavage to groups of 25 pregnant CD SD rats on days 6–15 of gestation at 0, 0.1, 3 or 21 mg/kg bw per day. Clinical observations were made throughout the study. Body weights were recorded on days 0, 6, 9, 12, 15, 18 and 20, and feed consumption was recorded on days 0–6, 6–9, 9–12, 12–15, 15–18 and 18–20 of gestation. Surviving dams were terminated on day 20 of gestation and necropsied; liver and uterine weights, the number of corpora lutea and uterine contents (implantation sites, resorptions, live and dead fetuses) were recorded. Fetuses were examined for visceral and skeletal malformations and variations.

There were no deaths. During the dosing period, clinical signs were observed at 21 mg/kg bw per day and included tremors, prone positioning, excitability, leaning/swaying and lower jaw movement; the tremors occurred within 10–60 minutes of dosing. Rats recovered following the cessation of

dosing. Lower mean body weight gain (~28%; $P < 0.01$) and feed consumption (11–16%; $P < 0.01$) also occurred at 21 mg/kg bw per day relative to the control group. There were no treatment-related macroscopic abnormalities or effects on gravid uterine or liver weight. There were no treatment-related effects on the number of resorptions, deaths, sex ratios or fetal body weight. Dichlorvos did not cause any malformations or variations. There was an increase in the incidence of male fetuses with variations at 21 mg/kg bw per day, with this result following a significant linear trend ($P < 0.05$); however, none of the pairwise comparisons were significantly different. In addition, no significant trend was observed for females or for all fetuses.

The NOAEL for maternal toxicity was 3 mg/kg bw per day, based on the occurrence of clinical signs and reduced body weight gain at 21 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 21 mg/kg bw per day, the highest dose tested (Tyl, Myers & Marr, 1991a).

Rabbits

Dichlorvos (purity 96.86%) was administered by gavage to pregnant rabbits (16 per group) on days 7–19 of gestation at 0, 0.1, 2.5 or 7 mg/kg bw per day. Clinical observations were made throughout the study. Body weights were recorded on days 0, 7, 12, 15, 19, 23 and 30 of gestation. Feed consumption was recorded on days 0–7, 7–9, 9–12, 12–15, 15–19, 19–23 and 23–30 of gestation. Dams were killed on day 30 of gestation (1.5 days before expected parturition) and necropsied; liver and uterine weights, the number of corpora lutea and uterine contents (implantation sites, resorptions, live and dead fetuses) were recorded. Fetuses were examined for visceral and skeletal malformations and variations.

Deaths occurred at 2.5 and 7 mg/kg bw per day (two and four dams, respectively). Two females from the 0.1 mg/kg bw per day group were removed from the study because of early delivery. At 7 mg/kg bw per day, clinical signs observed during gestation included ataxia (2–6 dams), increased respiration (2 dams), subdued behaviour (2 dams), rapid breathing (2–3 dams), salivation (2–3 dams), fine motor tremors (2 dams) and lurching (2 dams); these signs tended to occur in the same dams repeatedly over the dosing period. Whereas mean body weight gain at 2.5 and 7 mg/kg bw per day was approximately half that of the control group during the dosing period (days 7–19 of gestation), there were no statistically significant intergroup differences in body weight gain. At 7 mg/kg bw per day, mean feed consumption was 12% lower than in the control group during the dosing period, but was statistically significant ($P < 0.05$) only during days 7–9 of gestation. There was no treatment-related effect on gravid uterine weight or absolute liver weight. At 7 mg/kg bw per day, mean relative liver weight was approximately 12% lower than in the controls; although a significant dose-related, downward linear trend was noted, no significant differences were determined following pairwise comparisons. There were no treatment-related macroscopic abnormalities in dams. There were no effects on the number of resorptions, fetal deaths, sex ratio or fetal body weight. Dichlorvos did not cause any malformations or variations.

The NOAEL for maternal toxicity was 0.1 mg/kg bw per day, based on the occurrence of mortalities at and above 2.5 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 7 mg/kg bw per day, the highest dose tested (Tyl, Myers & Marr, 1991b).

2.6 Special studies

(a) Neurotoxicity

Hens

In an acute delayed neurotoxicity study, dichlorvos (purity 96.5%) in distilled water was administered by gavage to 10 fasted, adult domestic hens per group at 0 or 16.5 mg/kg bw. A positive control group was administered a gavage dose of tri-*o*-tolyl phosphate (TOCP) in corn oil at a dose

of 600 mg/kg bw. Atropine (5 mg/kg bw given at the time of dosing and 2 mg/kg bw thereafter as required) was given intramuscularly to dichlorvos-treated hens. The positive control group was sacrificed after 21 days, whereas the negative control and dichlorvos groups were redosed and observed for a further 21 days.

Lower limb weakness was observed in two control hens (days 7–13 and 22–24 in one hen; day 22 in the second hen). During forced locomotor activity, one control hen showed incoordination and pronounced ataxia on day 7 or 10, with two separate control hens refusing to hop or walk on day 25 or 36; neither was ataxic. No microscopic abnormalities of the brain, spinal cord or left/right sciatic nerves were observed in control hens. All TOCP-treated hens exhibited incoordination and lower limb weakness. During forced locomotor activity, slight to moderate ataxia was observed in two hens on days 3 and 7, respectively, whereas six hens exhibited slight to pronounced ataxia on day 21. Microscopic examination revealed degeneration of the sciatic nerve in five TOCP-treated hens.

Dichlorvos-treated hens displayed cholinergic signs approximately 30 minutes after dosing (lethargy or depression, incoordination, lower limb weakness, wing droop, reduced reaction to external stimuli, prostrate posture and loss of righting reflex). Two hens exhibited slight to moderate ataxia during forced locomotor activity (day 3) and refused to hop or walk. One of these hens was slightly ataxic on day 7 and refused to walk or hop on days 25, 36 and 39 when on its own, but appeared normal when in a group. Microscopic examination of this hen revealed swelling of the axis cylinder and sciatic nerve fibre degeneration, which was qualitatively similar to the sciatic nerve degeneration observed in the positive controls; no other microscopic abnormalities were observed in any other dichlorvos-treated hens. Slight ataxia was observed in one other hen on day 25 only. Two hens displayed lameness or a reluctance to walk (day 7) but were not ataxic, with one of these hens continuing to show lameness throughout the remainder of the study. The occurrence of neural degenerative changes in a single dichlorvos-treated hen is considered to show an equivocal relationship to treatment. Investigations of neuropathy target esterase (NTE) or brain ChE activity were not included (Beavers et al., 1988).

In a repeated-dose neurotoxicity study, dichlorvos (purity 97.87%) in distilled water was administered by gavage to adult domestic hens (21 per group) at 0, 0.3, 1 or 3 mg/kg bw per day for 28 days. Additional groups of three hens received dichlorvos at a dose of 0 or 0.1 mg/kg bw per day for analysis of brain ChE activity. A positive control group (n = 21) received TOCP in corn oil at 7.5 mg/kg bw per day. Hens were observed twice daily for 77 days (including the 28-day dosing period) and examined daily for signs of delayed locomotor ataxia. Body weights were recorded weekly. Three hens (two hens from the high-dose group) were sacrificed at 4 hours after the fourth dose (day 4) and 48 hours after the final dose (day 30) for measurement of brain ChE, brain NTE and spinal cord NTE activities. Postmortem examinations were performed on all hens. The brain, spinal cord and sciatic and tibial nerves were microscopically examined in hens sacrificed on days 49 and 77. No statistical analysis was performed.

Deaths occurred at 1 (one death on day 14) and 3 mg/kg bw per day (four deaths; two on day 3 and single deaths on days 27 and 29). At these same doses, clinical signs also occurred 30 minutes after dosing and included the inability to stand and unsteadiness (two hens at 1 mg/kg bw per day) or appearing subdued and unsteady (19/21 hens at 3 mg/kg bw per day). These signs persisted for 8 hours; survivors recovered by day 30. No clinical signs were observed in any other group, including the positive control. At 3 mg/kg bw per day, mean body weight gain was approximately 7% lower than in the controls over the dosing period, but had recovered by termination.

It was stated that there were no clinical signs of neurotoxicity and no evidence of delayed locomotor ataxia in any group, including the positive control; the results of the assessment of delayed locomotor ataxia were not provided, including results for the positive control. As TOCP did not induce clinical signs of delayed neurotoxicity, a number of supplementary studies were undertaken

Table 12. Cholinesterase inhibition in brain

Day	Mean % inhibition relative to the concurrent vehicle control group			
	0.1 mg/kg bw per day	0.3 mg/kg bw per day	1 mg/kg bw per day	3 mg/kg bw per day
4	—	7	44	63
30	0	26	35	54

From Redgrave (1994a)

by Redgrave (1994b); it was determined that the lack of an effect was due to substandard test material. There was a dose-related inhibition of brain ChE activity, which was toxicologically significant at and above 1 mg/kg bw per day at day 4 and at and above 0.3 mg/kg bw per day at day 30 (Table 12). There was no treatment-related effect on either brain or spinal cord NTE activities. In contrast, the positive control inhibited brain and spinal cord NTE activities relative to the negative control at day 4 (50% and 23%, respectively) and day 30 (61% and 55%, respectively).

There were no treatment-related macroscopic abnormalities. There were no treatment-related microscopic abnormalities of the brain, sciatic nerve or tibial nerve. The incidence of axonal degeneration of the spinal cord (predominantly graded as trace) was generally higher across all treated groups relative to the negative control (Table 13); in the absence of a dose–response relationship and as the incidence was within the historical control range of 20–80%, the occurrence of trace axonal degeneration was not considered treatment related. The incidence of minimal axonal degeneration in the upper and lower cervical spinal cord was slightly higher than the control (where no degeneration was evident) at 1 and 3 mg/kg bw per day; the incidence of these findings was only marginally above the performing laboratories' historical control range of 0–10%. The histopathology slides generated in this study were subsequently re-examined by Jortner (1994) and Hardisty (1998). Both evaluations concluded that there were no differences in the types or severity of lesions in the spinal cord of negative control and dichlorvos-treated hens across all doses. These re-evaluations concluded that dichlorvos did not cause organophosphate-induced delayed neuropathy (Redgrave, 1994a).

Rats

Dichlorvos (purity 97.87%) in deionized water was administered once by gavage to non-fasted SD Crl:CD BR rats (12 of each sex per group) at a dose of 0, 0.5, 35 or 70 mg/kg bw. Rats were observed daily for mortality and clinical signs for up to 14 days after dosing. Body weights were recorded on days −7, 0, 7 and 14. Rats found dead were weighed prior to necropsy. A functional observational battery (FOB) was performed on all rats 7 days prior to dosing and then at 15 minutes (the approximate time of peak effect), 7 days and 14 days after dosing. Decedents were necropsied. All survivors were sacrificed on day 14. Brain weights and dimensions were recorded. Any macroscopic abnormalities of the brain or spinal cord were noted. Histology was performed on the brain, spinal cord and peripheral nerves.

At 70 mg/kg bw, two males and five females died within 4 hours of dosing; the death of one of the males was attributable to an intubation error. Treatment-related FOB findings were recorded at the approximate time of maximal inhibition of ChE activity (~15 minutes after dosing) at doses of 35 and 70 mg/kg bw on day 0, but not on day 7 or 14. A summary of the main FOB findings is presented in Table 14. There were no treatment-related macroscopic or microscopic abnormalities, and there was no evidence of delayed neurotoxicity.

The NOAEL was 0.5 mg/kg bw, based on clinical signs of neurotoxicity (FOB) at and above the next highest dose of 35 mg/kg bw (Lamb, 1993a).

In a subchronic neurotoxicity study, dichlorvos (purity 97.87%) in water was administered by gavage to non-fasted SD Crl:CD BR rats (15 of each sex per group) at 0, 0.1, 7.5 or 15 mg/kg bw per

Table 13. Incidence of axonal degeneration in hens

Tissue site	Incidence of axonal degeneration									
	0 mg/kg bw per day		0.3 mg/kg bw per day		1 mg/kg bw per day		3 mg/kg bw per day		TOCP	
	Day 49	Day 77	Day 49	Day 77	Day 49	Day 77	Day 49	Day 77	Day 49	Day 77
Number of hens	*6*	*6*	*6*	*6*	*6*	*6*	*6*	*6*	*6*	*5*
Upper cervical spinal cord										
- total	1	5	6	3	4	5	6	4	5	6
- trace	1	5	6	3	3	4	4	3	5	0
- minimal	0	0	0	0	1	1	2	1	0	4
- moderate	0	0	0	0	0	0	0	0	0	2
Lower cervical spinal cord										
- total	1	1	4	5	4	6	4	4	5	3
- trace	1	1	4	5	4	5	3	3	4	3
- minimal	0	0	0	0	0	1	1	1	0	0
- moderate	0	0	0	0	0	0	0	0	1	0
Mid-thoracic spinal cord										
- total	1	5	4	3	4	3	4	5	6	6
- trace	1	5	3	3	4	2	4	5	5	4
- minimal	0	0	1	0	0	1	0	0	1	2
Lumbo-sacral spinal cord										
- total	0	0	3	4	2	3	3	2	5	5
- trace	0	0	3	4	2	3	3	2	3	3
- minimal	0	0	0	0	0	0	0	0	1	2
- moderate	0	0	0	0	0	0	0	0	1	0

From Redgrave (1994a)

day for 13 weeks. Ten and five rats of each sex per group were designated for ChE and neuropathological evaluation, respectively. Clinical signs were recorded daily, except on days when the FOB was performed. Rats were also observed at the time of peak effect (~15 minutes after dosing). Body weight and feed consumption were recorded weekly. FOB was performed on 10 rats of each sex per group pretreatment and then at weeks 4, 8 and 13 of dosing. Following the FOB, the same 10 rats of each sex per group were assessed for locomotor activity over 40 minutes. Rats found dead or sacrificed in a moribund condition were necropsied. Plasma and erythrocyte ChE activities were measured in the designated 10 rats of each sex per group at pretreatment and then during weeks 4, 8 and 13. At study termination, these same rats were sacrificed, and the following brain regions were weighed and analysed for total ChE activity (i.e. butyryl- and acetyl-): olfactory region, cerebellum, hippocampus, cerebral cortex, brainstem and midbrain. Whole brain weights and dimensions were recorded for rats designated for neuropathology.

One low-dose male was sacrificed in a moribund condition during week 12 as a result of mechanical trauma occurring during the FOB. There were no other deaths. Treatment-related clinical signs occurred at and above 7.5 mg/kg bw per day, but predominated at 15 mg/kg bw per day, and included cholinergic signs (hindlimb and forelimb tremors, repetitive jaw movements, salivation and lacrimation), the presence of a wet clear material on the forelimbs, rales, exophthalmos and chromodacryorrhoea. These occurred 15 minutes after dosing throughout the study. At 15 mg/kg bw per day, a small number of females (< 4) had a wet red, orange or yellow material and a dried red material

Table 14. Functional observational battery findings in rats (day 0)

Parameter	0 mg/kg bw		0.5 mg/kg bw		35 mg/kg bw		70 mg/kg bw	
	Males	Females	Males	Females	Males	Females	Males	Females
Number of rats	*12*	*12*	*12*	*12*	*12*	*12*	*10*	*7*
Autonomic effects								
- salivation	0	0	0	0	6*	6*	8*	7*
- pupil response absent	0	1	0	1	5*	5	8*	7*
- olfactory reaction absent	0	0	0	0	0	0	3	2
Neuromuscular effects								
- flattened extended limbs (home cage)	0	0	0	0	6*	4	8*	7*
- abnormal gait	0	0	0	0	10*	10*	9*	7*
- abnormal mobility	0	0	0	0	10*	9*	9*	7*
Central nervous system effects								
- tremors (home cage)	0	0	0	0	9*	9*	9*	7*
- tremors (open field)	0	0	0	0	10*	10*	9*	7*
Sensory observations								
- approach response absent	0	0	0	0	1	0	3	4*
- touch response absent	0	0	0	0	4	4	4*	5*
- tail response absent	0	0	0	0	7*	5*	9*	6*
Grip strength								
- forelimb	495	421	517	452	444	399	343**	271**
- hindlimb	369	331	331	311	356	297	267**	202**
- rotarod (s)	98	111	94	85	25	35	15**	5**
Physiological observations								
- catalepsy (s)	0.7	0.9	0.8	0.4	2.7	3.4	26.9**	10.3**
- temperature (°C)	38.8	39.4	38.4	39.4	36.5**	36.6**	35.7**	35.2**
Locomotor activity (counts)								
- total activity	1448	1842	1475	1680	654*	767*	725*	836*
- ambulatory activity	759	1017	819	864	317*	379*	393*	460*

From Lamb (1993a)

* $P < 0.05$ compared with the concurrent control (Fisher's exact test); ** $P < 0.01$ compared with the concurrent control (Dunnett's *t*-test)

around the mouth. The occurrence of salivation in a few animals at 0.1 mg/kg bw per day was not considered treatment related, as the incidence was comparable to that of the control group.

In males, body weight and body weight gain were unaffected by treatment. In females, mean body weight was significantly lower ($P < 0.05$; ~9%) than that of the controls at week 13, whereas body weight gain was significantly lower ($P < 0.05$ or 0.01) over the majority of the study. There was no treatment-related effect on feed consumption.

There were no treatment-related FOB findings assessed during weeks 4, 8 and 13, and there was no effect on motor activity.

Inhibition of plasma, erythrocyte and brain ChE activities occurred at 7.5 and 15 mg/kg bw per day, but did not exhibit an obvious dose–response relationship (Table 15); the statistical significance of these findings was variable. The inhibition of ChE activity in different brain regions was also variable and generally less than 20%.

Table 15. Inhibition of cholinesterase activity in rats

ChE	Mean % inhibition relative to the control					
	0.1 mg/kg bw per day		7.5 mg/kg bw per day		15 mg/kg bw per day	
	Males	Females	Males	Females	Males	Females
Plasma						
- week 3	0	0	37**	44**	34**	43**
- week 7	0	0	30	55*	35	51**
- week 13	0	0	42	56**	49	58**
Erythrocyte						
- week 3	14	0	23	14	35*	4
- week 7	6	20	12	38	8	42
- week 13	0	2	18	32	11	35
Brain						
- hippocampus	1	8	17	20	9	26
- olfactory	6	5	4	16	13	13
- midbrain	0	0	2	14	7	12
- brainstem	0	0	12	12	16*	10
- cerebellum	0	0	5	9	9	3
- cortex	0	0	12*	11**	15	10*

From Lamb (1993b)
* $P < 0.05$; ** $P < 0.01$

There were no treatment-related macroscopic abnormalities, including any effects on absolute or relative brain weights, the weight of specific brain regions or brain dimensions. There were no treatment-related microscopic abnormalities.

The NOAEL was 0.1 mg/kg bw per day, based on the occurrence of cholinergic signs and the inhibition of brain ChE activity at and above 7.5 mg/kg bw per day (Lamb, 1993b).

In a published in vitro study by Ehrich, Correll & Veronesi (1997), SH-SY5Y human and NB41A3 murine neuroblastoma cell lines were used to differentiate between neuropathic and acutely neurotoxic organophosphates (i.e. those inhibiting NTE and AChE activity, respectively). Concentration–response data were generated for dichlorvos (purity 96%; ethanol vehicle) and a number of other organophosphates following incubation with the two cell lines for 1 hour at 37 °C. Cytotoxicity was analysed using a neutral red assay. The results of these analyses are summarized in Table 16 and indicate that dichlorvos was a more potent inhibitor of AChE activity than of NTE activity. Under the conditions of this assay, the authors concluded that dichlorvos is unlikely to cause organophosphate-induced delayed neuropathy (and therefore more likely to cause acute cholinergic crisis via AChE inhibition) because the ratio of the median inhibitory concentration (IC_{50}) of NTE to that of AChE was large (> 40).

(b) Developmental neurotoxicity

Rats

In a range-finding study, groups of 15 pregnant Alpk:AP_fSD (Wistar-derived) rats were administered dichlorvos (purity 99%) in distilled water by gavage from day 7 of gestation to day 22 postpartum at a dose of 0, 0.1, 1 or 7.5 mg/kg bw per day. Observations for clinical signs were

Table 16. IC_{50} values for NTE and AChE inhibition by dichlorvos

Cell line	AChE[a]	NTE[a]	Ratio of IC_{50} NTE:AChE	Cytotoxicity[b]
SH-SY5Y cells (human)	0.35 µmol/l	20 µmol/l	42	1600 µmol/l
	(0.083–1.4 µmol/l)	(3.6–73 µmol/l)		[0.89]
	[0.96]	[0.93]		
NB41A3 cells (murine)	0.55 µmol/l	82 µmol/l	150	980 µmol/l
	(0.0031–11 µmol/l)	(61–440 µmol/l)		[0.68]
	[0.87]	[0.91]		

From Ehrich, Correll & Veronesi (1997)

[a] Results expressed as the IC_{50} (µmol/l), (confidence interval) and [r^2].

[b] Results expressed as the IC_{50} and [r^2].

made approximately 1 hour after dosing and at the end of each day. Body weight was recorded daily throughout the study, whereas feed consumption was recorded weekly during gestation. Five rats per group were sacrificed on day 22 of gestation and day 22 postpartum. Fetuses were removed from rats sacrificed on day 22 of gestation for analysis of erythrocyte and brain ChE activities. Macroscopic examinations were performed only on rats that died during the study. Pup numbers, sex, their clinical condition and body weights were recorded on days 1, 2, 5, 8, 15 and 22 postpartum. Litters were checked daily for mortalities. Five pups of each sex per group (generally one pup per litter) were sacrificed on days 2, 8, 15 and 22 postpartum for analysis of erythrocyte and brain ChE activities.

In maternal rats, there were no treatment-related mortalities or clinical signs and no reductions in body weight or body weight gain during gestation. At the highest dose, the mean body weight of maternal rats was 3–4% lower than that of the control group from day 11 postpartum; adjusted body weights (corrected for intergroup differences in pretreatment body weight) were significantly lower than the control body weights ($P < 0.05$) on all days except postpartum day 15. Feed consumption was comparable across all groups. One whole litter loss occurred at each of 0.1 and 1 mg/kg bw per day, but they were not considered treatment related in the absence of a dose–response relationship. There were no intergroup differences in gestation length, live pups at birth or litter size. At 1 mg/kg bw per day, the proportion of male pups was significantly higher ($P < 0.01$) than in the controls at days 1 and 2 postpartum, but was not considered treatment related in the absence of a dose–response relationship (51%, 46%, 65% and 46% at day 1 and 50%, 39%, 65% and 47% at day 2 at 0, 0.1, 1 and 7.5 mg/kg bw per day, respectively). At 1 and 7.5 mg/kg bw per day, the mean body weight of male pups was significantly lower ($P < 0.05$ and 0.01, respectively) than that of the controls on day 2 (6.9, 6.6, 6.5 and 6.4 g at 0, 0.1, 1 and 7.5 mg/kg bw per day, respectively), but, in the absence of similar differences on other days or in females, it was not attributable to treatment. There were no treatment-related clinical signs in pups.

In maternal rats, significant inhibition ($P < 0.01$) of erythrocyte ChE activity occurred at 1 and 7.5 mg/kg bw per day, whereas significant inhibition ($P < 0.01$) of brain ChE activity occurred at 7.5 mg/kg bw per day only (Table 17). There was little indication of any difference in brain or erythrocyte ChE activity from gestation day 22 to lactation day 22. In fetuses sacrificed on day 22 of gestation, significant inhibition ($P < 0.01$ or 0.05) of erythrocyte and brain ChE activities occurred at 7.5 mg/kg bw per day. In pups sampled during lactation, there was no significant inhibition of erythrocyte or brain ChE activities, indicating no toxicologically relevant exposure via the milk.

The NOAEL for maternal toxicity and embryo and fetal toxicity was 1 mg/kg bw per day, based on brain ChE inhibition at 7.5 mg/kg bw per day during gestation (Milburn, 2003b).

In the main developmental neurotoxicity study, groups of 30 pregnant Alpk:AP_fSD (Wistar-derived) rats were administered dichlorvos (purity 99.0%) in deionized water by gavage from day 7 of gestation to day 7 postpartum at a dose of 0, 0.1, 1 or 7.5 mg/kg bw per day. Offspring were dosed

Table 17. Inhibition of cholinesterase activity in maternal rats and their offspring

ChE	Mean % inhibition compared with the control group		
	0.1 mg/kg bw per day	1 mg/kg bw per day	7.5 mg/kg bw per day
Maternal			
Erythrocyte			
- gestation day 22	1	25**	48**
- lactation day 22	0	18	46**
Brain			
- gestation day 22	0	0	59**
- lactation day 22	0	12	67**
Fetus or pup (males/females)			
Erythrocyte			
- gestation day 22	14/10	0/14	28**/21
- lactation day 2	10/0	13/0	16/12
- lactation day 8	13/10	7/3	16/9
- lactation day 15	8/1	3/9	5/0
- lactation day 22	13/8	18/4	13/10
Brain			
- gestation day 22	9/4	0/0	16*/21**
- lactation day 2	7/0	6/0	4/3
- lactation day 8	8/8	3/0	8/4
- lactation day 15	0/0	0/0	4/0
- lactation day 22	5/1	5/0	7/0

From Milburn (2003b)
* $P < 0.05$; ** $P < 0.01$

similarly from postpartum days 8 to 22, weaned on postpartum day 29, then allowed to reach maturity (F_1 phase). Maternal rats were observed for clinical signs, with cage-side observations made twice daily. Body weight was recorded prior to dosing on gestation day 7 to lactation day 7, on lactation days 15 and 22, and at termination. A FOB was performed on days 10 and 17 of gestation and on days 2 and 9 of lactation. Litters were examined within 24 hours of parturition, with the sex, body weight and condition of each pup recorded on days 1 and 5 postpartum; daily observations for mortalities or abnormal pups were also made. On day 5 postpartum, litters with more than eight pups were standardized to eight randomly selected pups and designated as the F_1 generation. These pups were housed with their respective dam until day 29 postpartum. Pups not allocated to the F_1 generation were discarded. Maternal rats were sacrificed on day 29 postpartum.

F_1 pups were observed daily for clinical signs from day 5 postpartum, with any found dead or sacrificed in a moribund condition during the dosing period examined macroscopically. Body weights were recorded on days 5 and 8–22 postpartum (inclusive), then on days 29, 36, 43, 50, 57 and 63 (termination). F_1 males and females were examined from day 29 for preputial separation and vaginal opening, respectively, with body weight also recorded at this time. A FOB was performed on 10 rats of each sex per group on postpartum days 5, 12, 22, 36, 46 and 61 (one male or female per litter at 0, 0.1 and 1 mg/kg bw per day; one male and one female from some litters at 7.5 mg/kg bw per day). The following additional tests were made on one male or female from each litter: locomotor activity (days 14, 18, 22 and 60); auditory startle (days 23 and 61); and learning and memory (water maze) (days 24 and 59, with retesting on days 27 and 62, respectively). Any F_1 rats that died were necropsied. One female or one male per litter was sacrificed on days 12 and 63, and their brains

Table 18. Selected litter/pup parameters in developmental neurotoxicity study in rats

Parameter	0 mg/kg bw per day	0.1 mg/kg bw per day	1 mg/kg bw per day	7.5 mg/kg bw per day
Whole litter loss (%)	20	10	18	19
Live pups at birth (%)	99	99	94**	97
Litters with all pups born live (%)	83	93	78	74
Mean litter size at day 5 postpartum (pre-cull)	11.7	11.2	11.9	10.9
Pup survival from postpartum days 1 to 5 (%)	97	94	99	90**
Litters with pups found dead to day 5 postpartum	5	27	5	30

From Milburn (2003c)
** $P < 0.01$ compared with the concurrent vehicle control

were removed, weighed and processed for histopathology. The following tissues were also processed for histopathology: gastrocnemius muscle, eye (with retina and optic nerve), spinal cord, proximal sciatic nerve and distal tibial nerve. These tissues were microscopically examined in the control and high-dose groups only. No analysis of ChE activity was undertaken.

Maternal rats: A number of maternal rats were sacrificed prior to scheduled sacrifice for the following reasons: failure to litter (one and three rats at 1 and 7.5 mg/kg bw per day, respectively); total litter loss (six, three, five and five rats at 0, 0.1, 1 and 7.5 mg/kg bw per day, respectively); parturition difficulty (one rat at 1 mg/kg bw per day); clinical signs at day 3 postpartum (one high-dose rat); and insufficient pups (one, six, two and seven rats at 0, 0.1, 1 and 7.5 mg/kg bw per day, respectively). In the absence of dose–response relationships and the replication of these findings in the follow-up study, none were considered treatment related. There were no treatment-related clinical signs or effects on body weight. The FOB was unremarkable. Gestation length was comparable across all groups.

Pups: Selected litter/pup parameters are presented in Table 18. Relative to the control group, marginal reductions in the mean proportion of litters with all pups born live, litter size at day 5 postpartum and pup survival from postpartum days 1 to 5 ($P < 0.01$) were noted at the highest dose. The relatively small magnitude of these differences coupled with the absence of similar observations in the subsequent developmental neurotoxicity study at the identical dose (where in fact significant *increases* in these parameters were determined) indicates that these findings were not treatment related. There were no intergroup differences in sex ratios, mean body weight, total litter weight or the occurrence of clinical signs.

F_1 rats: There were no treatment-related clinical signs. Mean body weight was significantly higher (up to 7%; $P < 0.05$ or 0.01) than that of the control at 7.5 mg/kg bw per day, which was not considered adverse. The FOB was unremarkable. There was no treatment-related effect on the time of preputial separation or vaginal opening, with the body weight of high-dose females significantly higher than that of the control (~6%; $P < 0.05$) at the time of vaginal opening.

Motor activity was unremarkable. At 7.5 mg/kg bw per day, the mean maximum amplitude of the auditory startle response (V_{max}) of males was significantly higher ($P < 0.01$) than that of the controls on day 23 during repetitions 11–50 (but not 1–10), which was attributable to the higher body weight (12%) of this group relative to the controls. Additionally, there was no similar finding in females or on day 61 (in both sexes), no similar observation in the supplementary study that tested the same dose (Milburn, 2004) and no difference in the time to V_{max}. There was no treatment-related effect on learning or memory when these end-points were assessed via a Y-shaped water maze. Some statistically significant differences were noted at the highest dose when data were analysed as the mean percentage of successful trials (within a 3- to 10-second cut-off), but not as mean straight channel swim time or water maze time per trial; based on the inconsistency of these differences, they were

considered to be incidental findings. These conclusions are supported by observations at the same dose in the supplementary developmental neurotoxicity study (see below), in which no effects on locomotor activity, auditory startle or learning and memory occurred.

There was no treatment-related effect on absolute or relative brain or cerebellum weights in F_1 rats sacrificed at day 12 or 63. There were no treatment-related macroscopic abnormalities. Microscopic examination revealed a small increase in the incidence of demyelination of the distal and proximal tibial nerves (graded as minimal) and proximal sciatic nerve (graded as slight) in high-dose females compared with the controls (4/11 versus 2/12 rats, 7/11 versus 4/12 rats and 2/11 versus 0/12 rats, respectively). The incidences of these findings were within the historical control range for minimal to slight nerve demyelination (up to 70%) for this rat strain and are therefore not attributable to treatment.

Brain morphometric comparisons of high-dose rats with the controls showed no biologically relevant differences. Some statistically significant differences were noted for some brain measurements, but these showed a general lack of consistency between males and females, between rats sacrificed on days 12 and 63 and across other measurements of the same structure and were not corroborated by any histopathology or other evidence of developmental neurotoxicity. Further, the same differences were not determined in the supplementary study, which tested the identical dose. There was no treatment-related effect on the number of Purkinje cells.

The NOAEL for maternal toxicity, offspring toxicity and developmental neurotoxicity was 7.5 mg/kg bw per day, the highest dose tested (Milburn, 2003c).

A supplementary study was undertaken to confirm the findings of the main developmental neurotoxicity study because of the relatively high number of total litter losses. The conduct of the study was consistent with the main study except that rats were gavaged with dichlorvos at a dose of 0 or 7.5 mg/kg bw per day only. Fourteen control and six dichlorvos-treated maternal rats were sacrificed prior to scheduled termination for the following reasons: failure to litter (one control rat); clinical signs (one dichlorvos-treated rat; pale appearance and piloerection); total litter loss (five control rats and two dichlorvos-treated rats); and insufficient pups (eight control rats and three dichlorvos-treated rats).

There were no treatment-related clinical signs. With the exception of salivation in two dichlorvos-treated dams, the FOB was unremarkable. The mean body weight of dichlorvos-treated rats was approximately 3% higher than that of the control group on days 21 ($P < 0.05$) and 22 ($P < 0.01$) of gestation, with body weights remaining higher than those of the controls during lactation. However, there was no difference in body weight gain between the two groups during lactation. Gestation length was comparable between the control and treated groups. Differences in litter and pup parameters between the treated and control groups are summarized in Table 19. There were no adverse effects on any parameter, although it was noted that pup survival from day 1 to day 5 postpartum and total litter weight were significantly higher ($P < 0.01$) in the treated group compared with the control group. All other pup parameters were unremarkable.

In F_1 offspring, there were no treatment-related clinical signs or adverse FOB findings. The mean body weight of dichlorvos-treated rats was significantly higher (up to ~10%) than that of the control group from day 5 to day 36 postpartum in males ($P < 0.01$) and from day 5 to day 43 postpartum in females ($P < 0.01$ or 0.05). Body weights remained elevated for the duration of the study. In treated rats, the mean time of preputial separation or vaginal opening was significantly ($P < 0.05$), albeit slightly, shorter than the control (44.4 versus 43.7 days and 37.5 versus 36.4 days in the controls, respectively). The slightly earlier onset of maturity in dichlorvos-treated rats is likely attributable to the higher body weight. There were no treatment-related effects on motor activity, response to auditory startle or learning and memory.

Table 19. Selected litter/pup parameters in supplementary developmental neurotoxicity study in rats

Parameter	0 mg/kg bw per day	7.5 mg/kg bw per day
Pups born live (%)	95	98
Proportion of litters with all pups born live (%)	72	87
Pup survival (days 1–5) (%)	86	94**
Body weight		
- day 1 males/females	6.0/5.7	6.2/5.8
- day 5 males/females	9.3/9.0	10.1/9.5
Total litter weight (g)		
- day 1	66	70
- day 5	86	106**

From Milburn (2004)
** $P < 0.01$

Table 20. Microscopic nerve findings[a]

Parameter	Absolute number of rats (% incidence)			
	0 mg/kg bw per day		7.5 mg/kg bw per day	
	Males	Females	Males	Females
Number of rats	*11*	*12*	*11*	*12*
Distal tibial nerve				
- demyelination (minimal)	2 (18%)	1 (8%)	5 (45%)	5 (42%)
Proximal tibial nerve				
- demyelination (minimal)	6 (55%)	4 (33%)	8 (73%)	7 (58%)
- demyelination (slight)	0	1 (8%)	2 (18%)	2 (17%)
Proximal sciatic nerve				
- demyelination (minimal)	6 (55%)	4 (33%)	5 (45%)	5 (42%)
- demyelination (slight)	0	2 (17%)	2 (18%)	2 (17%)

From Milburn (2004)
[a] Historical control range of minimal to slight demyelination: distal tibial nerve, up to 40% in males and 60% in females; proximal tibial nerve, up to 80% in males and 60% in females; proximal sciatic nerve, up to 80% in males and 64% in females.

Consistent with the main study were small increases in the incidence of demyelination of the peripheral nerves in F_1 males and females (graded as minimal to slight), in particular the distal and proximal tibial nerves (Table 20). Sex-matched historical control data from the performing laboratory indicated that these incidences were within or marginally above the normal range for minimal to slight demyelination and on this basis are not considered biologically relevant.

Brain weights were unremarkable. In females sacrificed on day 12 postpartum, the mean absolute weight of the cerebellum was approximately 14% higher ($P < 0.01$) in dichlorvos-treated rats than in the controls. In the absence of a difference in males or on day 63, this difference was not considered treatment related. Morphometric analysis revealed a number of anatomical structures in the brain that were significantly different (in thickness, length or height) in dichlorvos-treated rats compared with the controls; more differences were observed in rats sacrificed on day 12 than on day 63. However, none of these findings could be attributed to treatment because of the lack of consistency over time or between sexes or because they were not corroborated by other measurements of the same

Table 21. Inhibition of cholinesterase activity in rats

ChE	% inhibition relative to the concurrent control group					
	2.1 mg/kg bw		5 mg/kg bw		35 mg/kg bw	
	Males	Females	Males	Females	Males	Females
Erythrocyte						
- day 1 (1 h post-dosing)	21**	18**	37**	38**	47**	47**
- day 8	14*	0	9	0	15	11
- day 15	0	0	0	0	0	7
Brain (cerebellum)						
- day 1 (1 h post-dosing)	12*	0	34**	17	64**	58*
- day 8	0	2	6	2	13	5
- day 15	11	7	9	6	18	4

From Twomey (2002a, 2003)
* $P < 0.05$; ** $P < 0.01$

structures. Historical control data were provided for some measurements, with these data confirming the absence of any biologically relevant differences. The number of Purkinje cells was comparable between treated and control rats.

Dichlorvos did not cause developmental neurotoxicity at a dose of 7.5 mg/kg bw per day (Milburn, 2004).

(c) Cholinesterase inhibition

Rats

A single dose of dichlorvos (unspecified purity) in deionized water was administered by gavage to Sprague-Dawley (Crl:CD(SD)IGS BR) rats (15 of each sex per group) at 0, 1, 5 or 35 mg/kg bw (the nominal dose of 1 mg/kg bw was analysed to be 2.1 mg/kg bw). The rats were then observed for up to 14 days. Observations for clinical signs were made throughout the study. Body weights were recorded weekly. On days 1 (1 hour after dosing), 8 and 15, five rats of each sex per group were sacrificed, and their brains were weighed and dissected for analysis of ChE activity in different regions (substrate unspecified); because of errors in dissection, only ChE activity in the cerebellum was reported. Blood was sampled for analysis of erythrocyte AChE activity.

One high-dose female died on the day of dosing, which was preceded by lacrimation, exophthalmos and salivation. On this same day, treatment-related clinical signs were observed in high-dose rats and included decreased activity, fasciculations, lacrimation, miosis, salivation and tremors. No further clinical signs occurred over the remainder of the study or in any other group. The only intergroup difference in body weight occurred in high-dose females on day 8, when mean body weight was slightly (~2%), but significantly, lower ($P < 0.05$) than in the control group. There were no intergroup differences in brain weight. Results of ChE activity measurements are presented in Table 21. On day 1, dose-related inhibition of erythrocyte and cerebellum ChE activities occurred. At every dose, significant inhibition of erythrocyte AChE activity occurred in both sexes. In males, significant inhibition of brain ChE activity occurred at every dose, whereas in females, significant inhibition was evident only at 35 mg/kg bw. No toxicologically significant inhibition of ChE activity occurred in rats sacrificed on day 8 or 15 (Twomey, 2002a, 2003).

Owing to the apparent discrepancy between the nominal low dose (i.e. 1 mg/kg bw) and the analytical low dose (i.e. 2.1 mg/kg bw), the previous study by Twomey (2002a) was repeated with

doses of 0 or 1 mg/kg bw. However, the study was terminated after day 1 due to the absence of clinical signs or inhibition of erythrocyte or brain (cerebellum) AChE activities (Twomey, 2002b).

In the third ChE inhibition study, dichlorvos (purity 99.0%) in deionized water was administered by gavage to Sprague-Dawley (Crl:CD(SD)IGS BR) rats (15 of each sex per group) at 0, 1 or 5 mg/kg bw, and then the rats were observed for up to 14 days. A separate group of nine males received 35 mg/kg bw, but additional dosing was suspended because of overt clinical distress. Additional groups of 15 rats were dosed with 0 or 15 mg/kg bw. Rats were observed throughout the study for clinical signs. Body weights were recorded weekly. One hour after dosing on day 1 and on day 8, five rats of each sex per dose were sacrificed, and their brains were weighed and dissected for analysis of AChE activity in different regions. Blood was sampled for analysis of erythrocyte AChE activity. On day 15, all remaining rats were sacrificed, and brain weights were recorded only for the control group; erythrocyte AChE activity was analysed only in the control and 5 mg/kg bw groups.

Four of the nine male rats dosed at 35 mg/kg bw were killed in moribund condition within an hour of dosing. Clinical signs observed in these and other rats in the group included decreased activity (five rats), fasciculations (eight rats), gasping (four rats), mydriasis (four rats), prostrate positioning (five rats), reduced righting reflex (two rats), reduced splayed reflex (two rats) and salivation (four rats). Survivors in this group either were sacrificed as scheduled, 1 hour after dosing (three rats), or had recovered by day 2. Clinical signs were also observed at 15 mg/kg bw (one female with fasciculations and miosis on the day of dosing) and 5 mg/kg bw (mydriasis in six males). No clinical signs occurred at 1 mg/kg bw or in the control group. There were no clinical signs observed in any group from days 2 to 15. Body weight was comparable across all groups. At day 1, toxicologically significant inhibition of AChE activity occurred in the majority of brain regions at and above 5 mg/kg bw in both sexes, which was coincident with the inhibition of erythrocyte AChE activity at the same doses (Table 22). The significant inhibition ($P < 0.05$) of AChE activity in the cortex of low-dose females on day 1, although at a level normally considered toxicologically significant, is considered an incidental finding, because it was not observed in males or in other brain regions of either sex and was not corroborated by the concomitant inhibition of erythrocyte ChE activity. Lower levels of inhibition occurred at day 8, indicating recovery.

The NOAEL was 1 mg/kg bw, based on the inhibition of AChE across all tested brain regions at the next highest dose of 5 mg/kg bw (Twomey, 2002c).

Dichlorvos (purity 99%) was administered as a single gavage dose of 0, 1, 5 or 15 mg/kg bw to groups of five male and five female Alpk:AP$_f$SD (Wistar-derived) rat pups that were 8, 15 or 22 days old. Pups were sacrificed approximately 1 hour after dosing (estimated time of peak effect) for analysis of erythrocyte and brain ChE activities (substrate not specified).

There were no deaths. Slight tremors occurred in one male and one female at the highest dose in 8- and 22-day-old pups; there were no other clinical signs. Results of ChE activity measurements are presented in Table 23. Dose-related inhibition of erythrocyte and brain ChE activities occurred in both sexes. Significant inhibition of brain ChE activity occurred at 5 and 15 mg/kg bw, with good corroboration with the inhibition of erythrocyte AChE activity; there was no difference between the three age groups. At the lowest dose, brain ChE activity was not inhibited; significant inhibition of erythrocyte AChE activity occurred in 8- and 15-day-old female pups; no inhibition occurred in 22-day-old female pups or in 8- or 22-day-old males.

The NOAEL was 1 mg/kg bw, based on the inhibition of brain ChE at and above 5 mg/kg bw (Moxon, 2002).

The recovery of ChE activity was examined following administration of dichlorvos (purity 99%; deionized water vehicle) to groups of 25 pre-weaning or adult female Alpk:AP$_f$SD (Wistar-derived)

Table 22. Inhibition of acetylcholinesterase activity in rats

AChE	% inhibition relative to the concurrent control group							
	1 mg/kg bw		5 mg/kg bw		15 mg/kg bw		35 mg/kg bw	
	Males	Females	Males	Females	Males	Females	Males	Females
Cerebellum								
- day 1 (1 h post-dosing)	0	14	17	18	—	52**	56**	—
- day 8	0	0	0	16	—	0	0	—
Cortex								
- day 1 (1 h post-dosing)	1	26*	35	37**	—	44*	76**	—
- day 8	0	20	0	4	—	4	0	—
Hippocampus								
- day 1 (1 h post-dosing)	6	0	26	28**	—	54**	51**	—
- day 8	0	15	0	9	—	10	0	—
Remainder of brain								
- day 1 (1 h post-dosing)	0	10	20	33	—	59**	69	—
- day 8	0	4	7	8	—	16	0	—
Half-brain								
- day 1 (1 h post-dosing)	0	18	27	34**	—	57**	75**	—
- day 8	0	0	10	0	—	7	24	—
Erythrocyte								
- day 1 (1 h post-dosing)	0	9	26*	29**	—	38**	40*	—
- day 8	7*	0	9*	2	—	9	15**	—

From Twomey (2002c)
—, not analysed; * $P < 0.05$; ** $P < 0.01$

Table 23. Inhibition of cholinesterase activity in pre-weaning rats

Parameter	Mean % inhibition relative to the concurrent control group					
	1 mg/kg bw		5 mg/kg bw		15 mg/kg	
	Males	Females	Males	Females	Males	Females
Erythrocyte AChE						
- 8-day-old pups	0	22*	26**	31**	62**	56**
- 15-day-old pups	9*	27**	29**	39**	53**	57**
- 22-day-old pups	0	0	35**	28**	49**	45**
Brain ChE						
- 8-day-old pups	0	0	26**	22**	65**	54**
- 15-day-old pups	0	0	28**	27*	61**	61**
- 22-day-old pups	0	0	31**	25**	58**	60**

From Moxon (2002)
* $P < 0.05$; ** $P < 0.01$

rats (15 and 42 days old, respectively) as an acute gavage dose of 0 or 15 mg/kg bw. At 1, 3, 8, 24 and 72 hours after dosing, five rats per group were sacrificed, and blood and brain were sampled for the analysis of erythrocyte and brain AChE activities, respectively.

There were no mortalities or clinical signs. Results of ChE measurements are summarized in Table 24. The highest level of inhibition of erythrocyte and brain AChE activities occurred at

Table 24. Recovery of cholinesterase activity in pre-weaning and adult rats

Time (h)	Mean % inhibition relative to the concurrent control group			
	Erythrocyte AChE		Brain AChE	
	Pre-weaning	Adult	Pre-weaning	Adult
1	53**	46**	59**	53**
3	52*	34**	51*	38*
8	27*	17	17	0
24	15	12*	12*	13*
72	0	15*	0	17

From Milburn (2003a)
* $P < 0.05$; ** $P < 0.01$

Table 25. Inhibition of acetylcholinesterase activity in pre-weaning and adult rats after seven daily doses

Parameter	Mean % inhibition relative to the concurrent control group					
	0.1 mg/kg bw		5 mg/kg bw		15 mg/kg	
	Males	Females	Males	Females	Males	Females
Erythrocyte AChE						
- 18-day-old pups	0	5	57**	58**	61**	65**
- 48-day-old rats	17*	11**	54**	54**	60**	53**
Brain AChE						
- 18-day-old pups	26**	24**	64**	61**	78**	74**
- 48-day-old rats	5	3	60**	54**	74**	72**

From Moxon (2004)
* $P < 0.05$; ** $P < 0.01$

1–3 hours; thereafter, the activities showed signs of recovery. By 72 hours, complete recovery was evident in pre-weaning rats, whereas residual inhibition was apparent in adults, but not at a level generally considered toxicologically significant (Milburn, 2003a).

Dichlorvos (purity 99%) was administered as seven consecutive gavage doses of 0, 0.1, 1, 5 or 15 mg/kg bw per day to groups of five male and five female Alpk:AP$_f$SD (Wistar-derived) rat pups (12 days old) or young adults (42 days old). Rats were sacrificed approximately 1 hour after the seventh dose (estimated time of peak effect) for the analysis of erythrocyte and brain AChE activities.

There were no deaths. In both age groups, slight tremors were observed in a single mid-dose male throughout the dosing period. At the highest dose, all rats displayed slight tremors following dosing. Results of AChE measurements are summarized in Table 25. Inhibition of erythrocyte and brain AChE activities occurred at 5 and 15 mg/kg bw per day in both age groups and sexes. At 0.1 mg/kg bw per day, significant inhibition of erythrocyte AChE activity (adult rats) or brain AChE activity (pups) was determined; the lack of inhibition of erythrocyte AChE activity in pups suggests that the apparent inhibition of brain AChE activity was not treatment related. It was noted that the concurrent control group had higher brain AChE activity compared with age-matched controls from other studies. An examination of these data indicated that for pre-weaning rats, the brain AChE activity in concurrent controls was 16–32% higher in males and 10–32% higher in females than for control groups from four other studies. When statistical comparisons were made with pooled control

data, no differences of statistical or toxicological significance were determined at 0.1 mg/kg bw. The NOAEL was therefore 0.1 mg/kg bw (Moxon, 2004).

(d) Mechanistic studies

In a published, non-guideline study, Ogutcu, Suludere & Kalender (2008) reported that antioxidants (vitamins C and E) reduced dichlorvos-induced hepatotoxicity in adult male Wistar rats (12 per group) following gavage dosing (in corn oil) at 0 or 1.6 mg/kg bw per day for 4 or 7 weeks. There were no deaths or reported clinical signs. At termination, mean body weight was approximately 7% and 13% lower ($P < 0.05$) than the starting weight in rats treated with dichlorvos for 4 or 7 weeks in the presence or absence of antioxidants, respectively. It was stated that feed intake was reduced in treated rats, but data were not presented. No analysis of ChE activity was undertaken. Mean absolute and relative liver weights were significantly higher ($P < 0.05$) in dichlorvos-treated rats (in either the presence or absence of antioxidants), but no histopathology was performed. Ultrastructural analysis of the liver by electron microscopy revealed mitochondrial swelling and dilatation of the endoplasmic reticulum; the toxicological significance of these observations is unclear. The authors' conclusion that dichlorvos was hepatotoxic was based solely on statistically significant differences (of a small magnitude) in a number of clinical chemistry parameters compared with the concurrent control group, with no consideration of the normal range of these parameters in age- and sexed-matched Wistar rats. A plausible mechanism for the unique occurrence of hepatotoxicity at this low dose was not discussed.

3. Observations in humans

3.1 Oral dosing studies in volunteers

In an open, non-randomized study by Gledhill (1997a,b), the effect of dichlorvos (purity 97.7%) on erythrocyte AChE activity was examined in six healthy male volunteers (20–32 years old; 66–89 kg bw). In the first phase of the study, 35 mg dichlorvos (0.40–0.53 mg/kg bw; mean of 0.5 mg/kg bw) in corn oil was ingested via a gelatine capsule (with 150 ml water) by four fasted subjects. This was followed 7 days later by the ingestion of a gelatine capsule containing only corn oil and then 7 days later with a second capsule containing 35 mg of dichlorvos. The two remaining subjects ingested only a single capsule containing 35 mg of dichlorvos. Blood was sampled prior to the first dose and at 1, 3, 5 and 7 days after each dose. Urine was also collected throughout the study with the aim of measuring the concentration of the dichlorvos metabolite, dimethyl phosphate. However, as a result of problems with the analytical method, no data were generated. There were no treatment-related adverse events or effects on body temperature. Following the first dose of dichlorvos, and relative to pretreatment activity, mean erythrocyte AChE inhibition was 7%, 0%, 10% and 0% at 1, 3, 5 and 7 days, respectively, after dosing. Following the second dose of dichlorvos, mean erythrocyte AChE inhibition was 4%, 5% ($P < 0.05$), 6% and 2% at 1, 3, 5 and 7 days, respectively, after dosing. None of these differences are considered toxicologically significant. The NOAEL was 0.5 mg/kg bw, based on the absence of erythrocyte AChE inhibition, adverse events or effects on body temperature at this dose.

In the second phase of the study, 21 mg dichlorvos (0.23–0.32 mg/kg bw, mean of 0.3 mg/kg bw) in corn oil was ingested via a gelatine capsule by the same six volunteers on a daily basis for 12 or 15 days (two and four subjects, respectively). Dosing was halted in four subjects on day 12 or 15 (two each) because they met the criterion of a greater than 20% inhibition of erythrocyte AChE activity for two successive samples. Blood was sampled immediately prior to and at 3, 5, 8, 10, 12, 15, 17, 19, 22, 26, 29, 33, 40, 47 and 54 days after the first dose. Urine was collected every 24 hours for 5 days following the first dose, then at 24 hours following the 10th dose. There were no treatment-related adverse events. Relative to pretreatment activity, mean inhibition of erythrocyte AChE

Table 26. Inhibition of erythrocyte acetylcholinesterase activity in male volunteers following repeated oral dosing

Day	Mean % erythrocyte AChE inhibition relative to pretreatment activity[a]
3	10
5	15*
8	**22***
10	**23***
12	**23***
15	**27***
17	**29***
19	**26***
22	**31***
24	**28***
26	**27***
29	**26***
33	19*
40	18
47	16
54	9

From Gledhill (1997a,b)
* $P < 0.05$
[a] Bolded values represent > 20% inhibition.

activity ranged from 9% to 31%; the mean level of inhibition was greater than 20% from days 8 to 29 and was statistically significant ($P < 0.01$ or 0.05) from days 5 to 33 (Table 26). An examination of individual AChE data revealed that all subjects attained a maximum level of inhibition of 28–39%, which is considered toxicologically significant. The recovery of erythrocyte AChE activity to near pretreatment levels was not achieved until day 54 (~40 days after the cessation of treatment). The lowest-observed-adverse-effect level (LOAEL) was 0.3 mg/kg bw per day, based on the inhibition of erythrocyte AChE activity (Gledhill, 1997a,b).

A single 70 mg dose of dichlorvos (purity 98%) formulated in corn oil was ingested by six healthy, fasted male volunteers (20–30 years old; 67–80 kg bw) in gelatine capsules with 150 ml water (0.88–1.04 mg/kg bw; mean of 0.94 mg/kg bw). Prior to dosing, baseline erythrocyte AChE activity was determined for each subject. Blood was sampled immediately prior to dosing, then on days 1, 3, 5 or 6, 7 and 14. Similar to the previous study, urine was collected at various times for the analysis of dimethyl phosphate. However, no data were generated because of analytical problems. Body temperature was recorded pre-dosing and at 2, 4, 8, 12 and 24 hours post-dosing.

There were no treatment-related adverse events, and there was no effect on body temperature. Mean inhibition of erythrocyte AChE activity was 6%, 4%, 10%, 12% and 11% of the mean pretreatment activity at 1, 3, 5, 7 and 14 days after dosing, respectively. These results were statistically significant ($P < 0.01$) for days 5, 7 and 14, but as the level of inhibition was less than or equal to 12%, none were considered toxicologically significant. An examination of individual subject data confirmed that no subjects had erythrocyte AChE inhibition above 18%, relative to their pretreatment activity. The NOAEL was 1 mg/kg bw, based on the absence of toxicologically significant erythrocyte AChE inhibition, clinical symptoms and effects on body temperature at this dose (Gledhill, 1997c,d).

Table 27. Inhibition of erythrocyte acetylcholinesterase activity in male volunteers following repeated oral dosing

Day	Mean % inhibition relative to pretreatment activity
1	1
2	5
4	5
7	9**
9	6
11	10**
14	14**
16	14**
18	16**
Post-dosing period (4–9 days after final dose)	17**

From Gledhill (1997e,f)
** $P < 0.01$ compared with the placebo

Dichlorvos (purity 98%), formulated in corn oil, was ingested by six healthy fasted male volunteers (19–34 years old; 61–89.7 kg bw) in gelatine capsules at 7 mg for 21 days (equal to 0.078–0.12 mg/kg bw per day; mean of 0.1 mg/kg bw per day) with 150 ml water. A control group of three healthy male subjects (31–33 years old; 74–83.5 kg bw) ingested 21 daily doses of the placebo (gelatine capsules containing corn oil) with 150 ml water. Baseline erythrocyte AChE activity was determined for each subject within 14 days prior to dosing. During the 21-day treatment period, blood was sampled on days 1, 2, 4, 7, 9, 11, 14, 16 and 18 and, post-dosing, on day 25 (four subjects), day 28 (three subjects) or days 29/30 (two subjects) for analysis of erythrocyte AChE activity. No explanation was given for the absence of the analysis of erythrocyte AChE activity on treatment days 18–21. Urine was collected at various times but was not analysed.

No treatment-related adverse events were evident. There was a time-related increase in the inhibition of erythrocyte AChE activity in dichlorvos-treated subjects (Table 27). Relative to pretreatment erythrocyte AChE activity, the mean level of inhibition ranged from 1% on day 1 to 16% on day 18; 17% inhibition was noted during the post-dosing period (days 25–30). Given the high statistical significance of these results ($P < 0.01$) and that the pattern of inhibition was consistent with the dosing regimen, the inhibition of erythrocyte AChE activity was clearly treatment related. An examination of individual subject data indicated that three of the six subjects treated with dichlorvos had single instances where the level of inhibition of erythrocyte AChE activity was above 20% (21–23% at day 18 or post-treatment). On this basis, a clear NOAEL was not established for the inhibition of erythrocyte AChE activity (Gledhill, 1997e,f).

3.2 Occupational exposure

The delayed-type allergenicity of seven pesticides (including dichlorvos) or chrysanthemum extracts (known to induce contact dermatitis due to their sesquiterpene lactones) was analysed in flower growers. Health information (e.g. previous pesticide use, history of allergies) was obtained by questionnaire. When subjects were patch tested with 0.02% aqueous dichlorvos, 6 of 59 males (10%) and 9 of 48 females (19%) exhibited a positive reaction (combined mean of 15%). Cross-reactivity with triforine was demonstrated (Ueda et al., 1994).

Mason (2000) examined the recovery of plasma and erythrocyte ChE activities in workers who had been exposed to dichlorvos. Subjects were involved in the production of dichlorvos vaporization

units and for a short period of time (duration unspecified) were potentially overexposed as a result of changes in production processes. The only biomonitoring of dichlorvos appeared to be in air, in which the mean concentration at the time was 1.15 mg/m^3. Blood was sampled from 20 workers for the analysis of plasma and erythrocyte ChE activities. Nineteen of the workers had plasma ChE inhibition greater than 30% of established baseline levels, whereas 13 of the workers had erythrocyte ChE inhibition of greater than 30%. The recovery of ChE activity was analysed in eight subjects who had the highest level of erythrocyte ChE inhibition (57–76%) on day 1. Plasma ChE activity showed an exponential pattern of recovery with a half-life of approximately 12 days, with recovery ostensibly complete by 50 days. Recovery of erythrocyte ChE activity was linear and completed by about 82 days.

A study was undertaken using data generated as part of the Agricultural Health Study (North Carolina and Iowa, USA), comprising a prospective cohort of 57 311 pesticide applicators, to investigate the association of parental pesticide exposure with the development of childhood cancer. Information on childhood cancer incidences for 17 357 children was provided by parents via questionnaires (1993–1997) and matched against cancer registry records. The odds ratio (OR) for dichlorvos was increased in fathers who had used the pesticide prenatally (OR = 2.06, 95% confidence interval [CI] = 0.86–4.90). However, as this result was based on small numbers (6 childhood cancer cases from 1218 paternally exposed pesticide applicators; 7% of the total cohort) and is not supported by other biological evidence, the authors did not consider the finding to be meaningful (Flower et al., 2004).

The cancer risk resulting from dichlorvos exposure was evaluated in a cohort of 4613 pesticide applicators enrolled in the Agricultural Health Study (see preceding study). These subjects were enrolled from 1993 to 1997 and followed for the development of cancer through 2004. Dichlorvos exposure was estimated using a detailed self-administered questionnaire. No evidence of an association between the lifetime risk of cancer and the use of dichlorvos was determined (Koutros et al., 2008).

The association of occupational exposure to a variety of pesticides (including dichlorvos) with the development of rhinitis was statistically analysed in private pesticide applicators enrolled in the Agricultural Health Study (see Flower et al., 2004). Polytomous and logistic regression models were used to assess this association while controlling for demographics and farm-related exposures. Rhinitis was self-reported by 67% of farmers. Only 3% of farmers with current rhinitis were actually exposed to dichlorvos, whereas 2% of farmers with no current rhinitis were exposed to dichlorvos. No significant association was determined using either the polytomous or dichotomous models. However, the authors stated that dichlorvos had the highest odds ratio (OR = 1.15, 95% CI = 1.03–1.28) in the dichotomous logistic model. The authors concluded that the uses of dichlorvos and three other organophosphorus pesticides were predictors of current rhinitis (Slager et al., 2010).

3.3 Poisoning case reports

Shimizu et al. (1996) analysed the tissue distribution of dichlorvos in a fatal poisoning case involving an elderly woman. The subject had apparently ingested 500 ml of a xylene formulation containing 75% dichlorvos. Autopsy at an unspecified time after death revealed congestion of the lungs and kidneys and a bleeding ulcer from the tongue to pharynx. Serum ChE activity was 2 IU/ml (normal range 206–459 IU/ml). The largest amount of dichlorvos was detected in the stomach contents (300 g total). The highest concentration (mg/ml or mg/kg) of dichlorvos was detected in the spleen (3340), followed by the heart (815), lung (81), kidney (80), blood (29), liver (20), brain (9.7) and urine (4.5).

The tissue distribution of dichlorvos was analysed in an elderly male who fatally ingested an unknown amount of a pesticide formulation containing dichlorvos (2% weight per volume [w/v]) and chlorpyrifos (5% w/v). The subject was autopsied approximately 28 hours after death. The lungs were oedematous, whereas the gastric mucosa was macerated and congested, with a large number of petechial haemorrhages. No dichlorvos was detectable in muscle, kidney, liver, lung, cerebrum, urine or blood collected from the heart, pulmonary artery and vein, or right femoral vein. Dichlorvos (mg/ml or mg/kg) was detectable in the stomach contents (2929 or 879 g in total), bile (9), spleen (0.54), pericardial fluid (0.44), vitreous humour (0.07), cerebrospinal fluid (0.03) and blood from the thoracic inferior vena cava (0.08) and thoracic aorta (0.04) (Moriya & Hashimoto, 1999).

Brahmi et al. (2004) reported four cases of extrapyramidal syndrome as a delayed but reversible complication in acute dichlorvos poisoning. In three cases, the amount of dichlorvos ingested was unknown, whereas in the fourth case, 100 ml of an unspecified formulation was ingested. In all cases, the patients presented in acute cholinergic crisis, with coma and respiratory failure; mechanical ventilation was required. The extrapyramidal syndrome (manifesting as tremor, hyperflexia, clonus, dystonia and rigidity) occurred within 5–15 days after exposure and lasted a few days to a few weeks. In all cases, recovery was complete. Limitations to this study identified by the authors were that the assessment of extrapyrimidal syndrome was undertaken retrospectively based on the patients' records and the possibility that atropine treatment confounded the result (by delaying the occurrence of the extrapyramidal symptoms).

The concentration of dichlorvos in various tissues was analysed in a deceased male who had deliberately ingested a xylene formulation of dichlorvos (purity 47.5%). The authors estimated that the ingested dose was 1000 mg/kg bw. Autopsy (conducted at an unspecified time after death) revealed diffuse congestion of the digestive tract and an ulcer at an unspecified site. Cardiac and peripheral blood, urine, heart, lung, kidney and liver were collected at an unspecified time after death. The concentrations (mg/l or mg/kg) of dichlorvos detected in these fluids/tissues were as follows (in order of highest to lowest): heart (1400), stomach contents (38), cardiac blood (4.4), lung (2), peripheral blood (1.3), urine (1.3) and kidney (1). No dichlorvos was detected in the liver (Abe et al., 2008).

Isolated bilateral vocal cord paralysis with intermediate syndrome was reported following acute cholinergic crisis in a 32-year-old woman who had deliberately ingested an unknown quantity of dichlorvos (unspecified purity). Following gastric decontamination, she was treated aggressively with atropine and required mechanical ventilation for about 4 days. Initial plasma ChE activity was 129 U/l (normal range 4100–9900 U/l), which increased to 1550 U/l by day 5. Cholinergic signs had resolved by 72 hours, but the subject reported progressive dyspnoea and dysphonia. Subsequent investigations found no peripheral nerve abnormalities, but determined vocal cord paralysis, which resolved by day 17 (Jin, Jeong & Lee, 2008).

Harputluoğlu et al. (2007) diagnosed acute pancreatitis, with subsequent pseudocyst development, in a 17-year-old female who had deliberately ingested two spoonfuls of a dichlorvos formulation (an emulsifiable concentrate containing dichlorvos at 550 g/l). Assuming that two spoonfuls equal at most 20 ml, the estimated dose for a 40 kg female would be 275 mg/kg bw. Clinical signs evident on admission (4 hours after ingestion) were blurred vision, abdominal pain, nausea and salivation. Her temperature was elevated (38.5 °C), and she had diffuse abdominal tenderness. She was treated with activated charcoal and atropine and eventually recovered. A number of clinical pathology parameters appeared elevated on the day of admission relative to measurements on days 2, 4 and 15 and week 4 (white blood cells, haematocrit, platelets, blood glucose, blood

urea nitrogen, serum amylase, serum lipase and C-reactive protein); however, no baseline values were given to assist in interpreting the toxicological relevance of the findings. The possibility that the pancreatitis was pre-existing cannot be ruled out, particularly as its apparent onset was rapid (within 4 hours of ingestion) and overt cholinergic stimulation was not observed; mechanistically, the occurrence of organophosphate-induced pancreatitis is thought to be due to prolonged hyperstimulation of acinar cells.

A case of necrotizing pancreatitis in a young male who deliberately ingested an unknown dose of dichlorvos was reported by Roeyen et al. (2008). Cholinergic signs were evident 3 hours after ingestion (sweating, vomiting). Haematocrit was normal, whereas serum amylase (1100 U/l; normal range 24–72 U/l) and lactate dehydrogenase (912 U/l; normal range 313–618 U/l) activities were elevated. Serum AChE activity was depressed (1330 U/l; normal range 5900–12 220 U/l). The patient was treated with atropine and pralidoxime. A computed tomography (CT) scan revealed acute pancreatitis. Haematocrit was slightly elevated above the normal range at 14 hours, with fever evident at 36 hours. C-reactive protein was 24 mg/dl (normal range < 0.5 mg/dl). An additional CT scan indicated that the tail of the pancreas had become necrotic. Histopathology confirmed the occurrence of (sterile) necrotizing pancreatitis. The patient subsequently recovered and was healthy at a 3-year follow-up.

Over a 3-month period, He et al. (2011) prospectively examined 41 patients with acute dichlorvos poisoning with particular emphasis on cardiac toxicity. The mean volume of dichlorvos (unspecified purity) ingested was 150 ml (range of 50–350 ml), and the mean interval between poisoning and hospital admission was 21 hours (range of 3–120 hours). All patients were treated with atropine and pralidoxime chloride and mechanically ventilated. Each patient's clinical condition was closely monitored during hospitalization. Blood was sampled on hospital days 1, 3 and 5 and at discharge for the analysis of AChE activity, epinephrine and norepinephrine. Transthoracic echocardiography and myocardial single-photon emission computed tomography (SPECT) imaging were performed on patients in the acute phase of poisoning, at discharge and during a follow-up examination.

Survival was approximately 90%. Clinical signs recorded on the day of admission included cholinergic signs (incontinence, 85%; salivation and sweating, ~40%; myosis and hypotension, ~15%; loose stools, ~12%; vomiting, frothy sputum, lacrimation, bradycardia, all < 10%), nicotinic effects (fasciculations, 100%; tachycardia, ~90%; hypertension, ~10%) and central nervous system effects (spasm/convulsion, 61%; coma, 100%). The main electrocardiogram findings on admission were sinus tachycardia (> 100 beats/minute) (100%) and ST-T changes (~68%), including ST elevation (37%) and ST depression (31%). Echocardiographic and SPECT analysis revealed reversible myocardial dysfunction, including decreased wall motion of the interventricular septum and left ventricle (acute phase only) and abnormal left ventricle perfusion. Mean AChE activity was 1232, 1805, 3842 and 6693 IU/l (normal range = 7000–10 000 IU/l) on days 1, 3 and 5 and at discharge, respectively. Serum acetylcholine, epinephrine and norepinephrine levels peaked on day 1 but recovered thereafter. Mean serum creatinine kinase myocardial isozyme level was 12.5, 29, 10 and 2.75 ng/ml (normal range < 3.6 ng/ml) on days 1, 3 and 5 and at discharge, respectively. Mean cardiac troponine I levels followed a pattern of increase and recovery similar to that of creatinine kinase, with mean levels of 1, 6, 2 and 0.12 ng/ml (normal range < 0.05 ng/ml) on days 1, 3 and 5 and at discharge, respectively. The study authors identified a number of limitations to the study, including its conduct at a single centre and the absence of an anatomical examination of the four patients who died; the authors concluded that studies involving more subjects at multiple centres are required to further investigate the cardiotoxicity of dichlorvos.

Comments

Biochemical aspects

Following oral dosing with ^{14}C-labelled dichlorvos, similar patterns of excretion of radioactivity were observed in mice, rats, hamsters and humans. Excretion was also similar in male and female rats and following both oral and intravenous dosing. Recovery of radioactivity was greater than 90%, with the majority excreted within 24 hours of dosing. The main excretion pathways of radioactivity were via carbon dioxide (30% in mice and humans, 50% in hamsters, up to 60% in rats) and urine (30% in mice, up to 17% in rats, 20% in hamsters and 8% in humans). Relatively low levels of radioactivity were detected in faeces (3% in mice, 5% in hamsters and 13% in rats). The detection of radioactivity in the carcass of mice (30%), rats (26%) and hamsters (15%) is likely due to the incorporation of ^{14}C into protein. Based on the level of radioactivity in carbon dioxide, urine, the carcass and tissues following oral dosing, absorption was estimated to be 92–95% in rats. Analysis of urine identified similar levels of hippuric acid in mice, hamsters and humans (< 1% of the administered dose); desmethyl dichlorvos was detected in mouse and human urine at ~19% and 0.2%, respectively; and urea was detected at concentrations below 1% in both mouse and human urine. In rats, the levels of hippuric acid and urea were less than 6% and 3–30% of total faecal radioactivity and 4–24% and 19–33% of total urinary radioactivity, respectively; no other metabolites were identified in excreta, which may be due to their volatility or degradation. In rats, approximately 6–13% of urinary metabolites were glucuronidated.

The in vitro half-life of dichlorvos in human blood was less than 15 minutes at 37 ºC.

The level of dermal absorption in rats was 22–30%, which occurred within 10 hours of exposure.

Toxicological data

As with other organophosphorus insecticides, inhibition of ChE activity is the most sensitive toxicological end-point following acute or repeated exposures to dichlorvos.

Dichlorvos has marked acute oral toxicity. In acute oral dosing studies, clinical signs and deaths occurred rapidly in rats and rabbits. Consistent with the cholinergic effects observed with other organophosphorus compounds, signs of acute intoxication with dichlorvos included salivation, lacrimation, dyspnoea and tachypnoea (muscarinic effects), muscle tremors, clonic–tonic spasms, lethargy, paresis, splayed gait, prostration/lateral positioning (nicotinic effects), and restlessness, ataxia and coma (central nervous system effects).

The results of acute toxicity studies evaluated by the current Meeting were consistent with the acute toxicity profile of dichlorvos established by previous Meetings. The oral LD_{50} in rats was 57–108 mg/kg bw, whereas the oral LD_{50} in rabbits was 74 mg/kg bw. The dermal LD_{50} in rats was 210 mg/kg bw. In rats and mice, LC_{50} values were 0.23 and greater than 0.22 mg/l, respectively, for head-only exposure to dichlorvos aerosols. It was not possible to determine the skin and eye irritancy potential of dichlorvos because of high levels of toxicity in the study animals. In a non-guideline study, dichlorvos was classifiable as a skin sensitizer in guinea-pigs (maximization test).

The main toxicological findings in repeated-dose studies in rats and dogs were inhibition of ChE activity and, at higher doses, reduced body weight gain and signs of neurotoxicity. In short-term studies of toxicity of less than 12 months' duration, the NOAEL for inhibition of erythrocyte AChE activity was 0.1 mg/kg bw per day in rats and 0.05 mg/kg bw per day in dogs. The NOAEL for inhibition of brain ChE activity was 1.5 mg/kg bw per day in rats and 0.05 mg/kg bw per day in dogs. Toxicity observed in rats and dogs was limited to the characteristic muscarinic signs (salivation or vomiting) and reduced body weight gain. The effect doses for these clinical signs in short-term studies correlated with moderate levels of inhibition of brain ChE activity (up to ~50%).

Previous Meetings have evaluated more than 10 carcinogenicity studies conducted in mice and rats that received dichlorvos orally (diet, drinking-water or gavage) or by inhalation. The majority of the oral dosing studies and all of the inhalation studies found no evidence of carcinogenicity. The 1993 Meeting concluded that the occurrence of a small number of forestomach lesions in B6C3F1 mice (papillomas) in a United States National Toxicology Program study was attributable to the localized effect of dichlorvos administered by corn oil gavage. The 1993 Meeting also concluded that exposure to dichlorvos would not result in chronic human health hazards at doses below those that result in AChE inhibition.

No new long-term studies of toxicity or carcinogenicity were considered by the current Meeting. Two drinking-water studies conducted in mice and rats were resubmitted, as the studies now had an improved English translation and had been statistically reanalysed by the authors. Dichlorvos was not carcinogenic under the conditions of either study. In the mouse study, observations of squamous cell hyperplasia, with apparent progression to papillomas in males, suggested treatment-related proliferative changes in the glandular region of the stomach. However, the same findings were not observed in females and were not corroborated by findings from a gavage study using the same mouse strain in which papillomas were observed in the forestomach of females.

Numerous in vitro and in vivo experiments have tested the genotoxic potential of dichlorvos. The 1993 Meeting concluded that dichlorvos and its major metabolite, dichloroacetaldehyde, had been adequately tested in in vitro and in vivo genotoxicity assays. Unpublished genotoxicity studies evaluated by the current Meeting indicated that dichlorvos was mutagenic to mouse lymphoma cells in vitro (the mutation frequency higher in the absence of exogenous metabolic activation), whereas five unpublished in vivo assays detected no evidence of genotoxicity (mouse dominant lethal assay, mouse chromosomal aberration assay in bone marrow and spermatocytes, sister chromatid exchanges in mice and mouse micronucleus test). Published studies reported a genotoxic response for a number of in vitro end-points, including mutations, chromosomal aberrations, micronuclei, sister chromatid exchanges and DNA damage. In those published in vivo studies considered suitable for regulatory purposes, dichlorvos was not genotoxic. The consistently negative in vivo genotoxicity response can be attributed to the rapid metabolism of dichlorvos, which limits systemic exposure to intact dichlorvos at concentrations likely to lead to direct interactions with DNA. The occurrence of mutations in the liver of transgenic mice administered repeated intraperitoneal doses is consistent with a mechanism of genotoxicity resulting from high localized tissue concentrations of unmetabolized dichlorvos; in humans, scenarios of prolonged systemic exposure to unmetabolized dichlorvos are highly unlikely.

The Meeting noted the weight of evidence from previously considered carcinogenicity studies, which indicated that dichlorvos possesses no systemic genotoxic potential. Further, the 1993 Meeting noted that dichlorvos methylated DNA in vitro at a rate that is 8–9 orders of magnitude lower than the rate of phosphorylation. Therefore, DNA alkylation is unlikely to occur at doses of dichlorvos that are not inhibitory to erythrocyte/brain ChE activities.

The Meeting concluded that dichlorvos is unlikely to be genotoxic in vivo.

In the absence of an in vivo genotoxic response and any carcinogenic response relevant to humans, the Meeting concluded that dichlorvos is unlikely to pose a carcinogenic risk to humans.

In a two-generation study of reproductive toxicity in rats, in which exposure was via the drinking-water, treatment-related effects included the inhibition of ChE activity and reduced body weight gain. The inhibition of brain ChE activity in parental males and females (up to 50% at the highest dose) was not associated with cholinergic signs. The NOAEL for parental toxicity was 0.5 mg/kg bw per day, based on the inhibition of brain ChE activity at 2 mg/kg bw per day. The NOAEL for offspring toxicity was 2 mg/kg bw per day, based on lower pup weights in both generations at 8 mg/kg bw per day. The NOAEL for reproductive toxicity was 2 mg/kg bw per day, based on reduced fertility and pregnancy indices, increased stillbirths in the F_2 generation and abnormal cycling in F_1 maternal rats at 8 mg/kg bw per day.

Non-guideline studies reported effects on rat sperm following repeated gavage doses of 2–10 mg/kg bw per day, but these were considered to be of questionable biological relevance due to methodological limitations.

In an in vitro assay, dichlorvos did not bind to the human or mouse estrogen receptor and bound with only very low affinity to the human and mouse androgen receptors.

In studies of developmental toxicity with dichlorvos following gavage dosing, teratogenicity was not observed at doses up to 21 and 7 mg/kg bw per day in rats and rabbits, respectively. Maternal toxicity, including cholinergic signs and deaths, was observed at lower doses. In rats, the NOAEL for maternal toxicity was 3 mg/kg bw per day, based on the occurrence of clinical signs (tremors and prone positioning) and reduced body weight gain at 21 mg/kg bw per day. In rabbits, the NOAEL for maternal toxicity was 0.1 mg/kg bw per day, based on the occurrence of deaths at 2.5 mg/kg bw per day and above. It was noted that these deaths occurred at doses lower than the oral LD_{50} for rabbits.

The Meeting concluded that dichlorvos did not cause developmental toxicity and that it was not teratogenic.

In studies of delayed neurotoxicity, dichlorvos was administered by gavage to hens either as a single dose of 16.5 mg/kg bw or as repeated doses of up to 3 mg/kg bw per day for 28 days; there was no evidence of delayed neuropathy. The previous Meeting noted that dichlorvos caused delayed polyneuropathy in hens at doses much higher than the LD_{50}, and cases of delayed polyneuropathy were reported in humans following severe, life-threatening intoxications. A supplementary in vitro study confirmed that dichlorvos is a more potent inhibitor of AChE activity than of NTE activity. The Meeting concluded that dichlorvos can cause delayed polyneuropathy in humans, but only after acute poisoning causing a severe cholinergic syndrome that would be lethal if not properly treated.

In studies of neurotoxicity in rats, dichlorvos was administered as a single dose of up to 70 mg/kg bw or as repeated doses of up to 15 mg/kg bw per day. The NOAEL following a single gavage dose was 0.5 mg/kg bw, based on clinical signs of neurotoxicity at 35 mg/kg bw observed during the functional observational battery 15 minutes after dosing; no signs of neurotoxicity were observed 7 or 14 days after dosing. Following repeated gavage doses of up to 15 mg/kg bw per day for 13 weeks, clinical signs of neurotoxicity were observed within 15 minutes of dosing throughout the study, at and above 7.5 mg/kg bw per day. These signs coincided with the inhibition of ChE activity in erythrocytes and brain.

Dichlorvos did not cause developmental neurotoxicity following repeated gavage doses of up to 7.5 mg/kg bw per day. In the range-finding study, inhibition of brain ChE activity occurred in dams (~60%) and pups (~20%) during gestation only, in the absence of clinical signs. In the main and supplementary studies, in which no analysis of ChE activity was undertaken, the NOAEL for maternal and offspring toxicity was 7.5 mg/kg bw per day, the highest dose tested.

In studies investigating the inhibition of ChE activity in rats following an acute gavage dose up to 35 mg/kg bw, the NOAEL for the inhibition of brain AChE activities was 1 mg/kg bw. At the next highest dose of 5 mg/kg bw, inhibition of erythrocyte and brain AChE activities co-occurred (~30%), whereas clinical signs were not observed until the level of inhibition reached approximately 50% (at and above 15 mg/kg bw). There was no difference in erythrocyte and brain ChE inhibition between rat pups of different ages or between rat pups and adults. Following an acute gavage dose of dichlorvos of 15 mg/kg bw, maximum inhibition of erythrocyte and brain ChE activities was measured at 1–3 hours after dosing, with recovery apparent from 8 hours post-dosing. This observation is consistent with the half-life of spontaneous reactivation of erythrocyte AChE activity reported in previous monographs of approximately 2 hours; in comparison, the half-life of reactivation of human erythrocyte AChE activity is approximately 15 days. Following 7 consecutive gavage doses of up to 15 mg/kg bw per day, inhibition of erythrocyte and brain AChE activities occurred at and above 5 mg/kg bw per day; the NOAEL was 0.1 mg/kg bw per day.

The Meeting considered new studies in male volunteers in which dichlorvos was ingested in gelatine capsules either as an acute dose or as short-term repeated doses. No inhibition of erythrocyte AChE activity occurred in six volunteers following a single dose of 0.5 mg/kg bw. These same six volunteers then ingested 0.3 mg/kg bw per day for 12 or 15 days. However, dosing was stopped because inhibition of erythrocyte AChE activity exceeded 20% in four subjects (the mean maximum level of inhibition was ~30%); the NOAEL was less than 0.3 mg/kg bw per day. The recovery of erythrocyte AChE activity to near pretreatment levels occurred approximately 40 days after the cessation of treatment. In a second acute dose study conducted in a different group of six volunteers, the NOAEL was 1 mg/kg bw, based on the absence of erythrocyte AChE inhibition, adverse events or effects on body temperature at this dose. In a 21-day study conducted in a different six volunteers at a dose of 0.1 mg/kg bw per day, there was a time-related decrease in erythrocyte AChE activity, which reached a mean of 16% on day 18. Although dosing continued for a further 3 days, AChE activity was not analysed again until 4–9 days after the final dose, and therefore there is some uncertainty about whether steady state had been fully reached. Further, three of the six volunteers had a greater than 20% level of erythrocyte AChE inhibition on day 18 or during the post-treatment period, and on this basis, the Meeting concluded that a clear NOAEL had not been demonstrated.

In an occupational study, 15% of flower workers (males and females) tested positive for dichlorvos in a skin patch test.

In workers who were exposed to dichlorvos for short periods of time during the manufacture of vaporization units, recovery of plasma and erythrocyte ChE activities took approximately 50 and 82 days, respectively.

Epidemiological studies provided no evidence for the association of parental pesticide exposure with the development of childhood cancer (cohort of 1218) or the lifetime risk of cancer in pesticide applicators (cohort of 4613).

Case reports provided additional clinical observations in humans following acute cholinergic crisis. These observations included four cases of delayed extrapyramidal syndrome, isolated bilateral vocal cord paralysis with intermediate syndrome and two cases of pancreatitis, one with the possible development of a pseudocyst. In a study involving 41 severely poisoned patients, dichlorvos caused reversible myocardial dysfunction.

In three separately reported cases of fatal ingestion, dichlorvos was detected in various human tissues. However, a meaningful comparison between the cases was difficult because of differences in the ingested dose and sampling interval. A relatively high concentration of dichlorvos was uniquely detected in the spleen or heart in separate cases, whereas the majority of dichlorvos was detected in the stomach contents. Relatively low concentrations were detected in the liver, brain, blood and urine.

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

Toxicological evaluation

The Meeting confirmed the current ADI of 0–0.004 mg/kg bw based on the NOAEL of 0.04 mg/kg bw per day for the inhibition of erythrocyte AChE activity in a 21-day study in male volunteers (Annex 5, reference *70*). The ADI was previously based on the NOAEL of 0.033 mg/kg bw per day in a 28-day study in male volunteers for the same end-point (Annex 1, reference *9*) and before that on the NOAEL of 0.37 mg/kg bw per day in a 90-day study in dogs for the inhibition of brain ChE activity (Annex 1, reference *7*).

The Meeting considered two new studies conducted in male volunteers at doses higher than those tested in the two pivotal human studies underpinning the current ADI. Neither study was considered a suitable basis for an ADI, because clear NOAELs had not been demonstrated. The Meeting

considered the ADI to be protective for other, non-neurotoxic effects of dichlorvos observed in short- and long-term studies with repeated doses and in studies of reproductive and developmental toxicity, where the use of an interspecies safety factor of 10 would be appropriate. The absence of any age- or sex-specific differences in ChE inhibition in rats confirmed the current ADI to be protective of the entire population.

The Meeting established an acute reference dose (ARfD) of 0.1 mg/kg bw, based on the NOAEL of 1 mg/kg bw for erythrocyte AChE inhibition in the acute oral study in male volunteers and using a 10-fold intraspecies safety factor. The NOAEL is supported by observations in two other volunteer studies in which no erythrocyte AChE inhibition occurred 1 day after dosing at 0.5 and 0.1 mg/kg bw, respectively.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Rat	Acute toxicity study[a]	Toxicity (inhibition of brain ChE activity)	1 mg/kg bw	5 mg/kg bw
	Acute neurotoxicity study[a]	Toxicity (clinical signs)	0.5 mg/kg bw	35 mg/kg bw
	Developmental toxicity study[a]	Maternal toxicity	3 mg/kg bw per day	21 mg/kg bw per day
		Embryo and fetal toxicity	21 mg/kg bw per day[b]	—
	Developmental neurotoxicity study[a]	Developmental neurotoxicity, maternal toxicity and offspring toxicity	7.5 mg/kg bw per day[b]	—
	Thirteen-week toxicity study[c]	Toxicity (inhibition of brain ChE activity and clinical signs)	1.5 mg/kg bw per day	15 mg/kg bw per day
	Thirteen-week neurotoxicity study[a]	Toxicity (inhibition of brain ChE activity and clinical signs)	0.1 mg/kg bw per day	7.5 mg/kg bw per day
	Two-generation reproduction study[d]	Reproductive toxicity	2 mg/kg bw per day	8 mg/kg bw per day
		Parental toxicity	0.5 mg/kg bw per day	2 mg/kg bw per day
		Offspring toxicity	2 mg/kg bw per day	8 mg/kg bw per day
Rabbit	Developmental toxicity study[a]	Maternal toxicity	0.1 mg/kg bw per day	2.5 mg/kg bw per day
		Embryo and fetal toxicity	7 mg/kg bw per day[b]	—
Dog	One-year toxicity study[e]	Toxicity (inhibition of brain ChE activity and clinical signs)	0.05 mg/kg bw per day	1 mg/kg bw per day
Human	Acute toxicity study[e]	Toxicity (inhibition of erythrocyte AChE activity)	1 mg/kg bw[b]	—
	Twenty-one-day toxicity study[e]	Toxicity (inhibition of erythrocyte AChE activity)	—	0.1 mg/kg bw per day
	Twenty-one-day toxicity study[e,f]	Toxicity (inhibition of erythrocyte AChE activity)	0.04 mg/kg bw per day[b]	—
	Twenty-eight-day toxicity study[e,g]	Toxicity (inhibition of erythrocyte AChE activity)	0.033 mg/kg bw per day[b]	—

[a] Gavage administration.
[b] Highest dose tested.
[c] Dietary administration.
[d] Administration in drinking-water.
[e] Administration in capsules.
[f] Evaluated previously (Annex 1, reference *70*).
[g] Evaluated previously (Annex 1, reference *9*).

Estimate of acceptable daily intake for humans

0–0.004 mg/kg bw

Estimate of acute reference dose

0.1 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 exposures

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

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid (T_{max} < 0.5 h) and essentially complete (92–95% in rats)
Dermal absorption	22–30% within 10 h (rats)
Distribution	Distributes to most tissues; highest levels of radiolabel detected in the carcass and liver, with lower levels in the blood and kidneys
Potential for accumulation	Low; no evidence of accumulation
Rate and extent of excretion	Rapid (within 24 h) and extensive excretion of radiolabel (mainly via carbon dioxide and urine)
Metabolism in animals	Extensive by hydrolysis and demethylation (in vitro half-life in human blood < 15 min)
Toxicologically significant compounds (animals, plants and the environment)	Dichlorvos, dichloroacetaldehyde
Acute toxicity	
Rat, LD_{50}, oral	57–108 mg/kg bw
Rat, LD_{50}, dermal	210 mg/kg bw
Rat, LC_{50}, inhalation	0.23 mg/l (4 h, head-only exposure)
Rabbit, dermal irritation	Not assessed due to high toxicity
Rabbit, ocular irritation	Not assessed due to high toxicity
Human, skin sensitization (skin patch test)	Skin sensitizer
Short-term studies of toxicity	
Target/critical effect	Cholinesterase inhibition
Lowest relevant oral NOAEL	0.05 mg/kg bw per day (dogs)
Lowest relevant dermal NOAEL	No new data
Lowest relevant inhalation NOAEC	No new data
Genotoxicity	
	Not genotoxic in vivo following oral dosing
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Cholinesterase inhibition
Lowest relevant oral NOAEL	No new data
Carcinogenicity	Unlikely to pose a carcinogenic risk to humans

Reproductive toxicity	
Reproduction target/critical effect	Reduced fertility and pregnancy indices, increased stillbirths and abnormal cycling in maternal rats
Lowest relevant reproductive NOAEL	2 mg/kg bw per day (rats)
Developmental target/critical effect	No developmental toxicity, including teratogenicity (rats, rabbits)
Lowest relevant developmental NOAEL	21 mg/kg bw per day (rats), 7 mg/kg bw per day (rabbits); highest doses tested
Neurotoxicity/delayed neurotoxicity	
Neurotoxicity	Neurotoxic due to cholinesterase inhibition
	No evidence of delayed neuropathy up to 16.5 mg/kg bw (hens) or 70 mg/kg bw (rats), the highest doses tested
	Very weak inhibitor of NTE activity in vitro
Lowest relevant oral NOAEL	0.1 mg/kg bw per day (13-week rat study)
Developmental neurotoxicity	No evidence of developmental neurotoxicity up to 7.5 mg/kg bw per day (rats), highest dose tested
Medical data	
	No epidemiological evidence of increased cancer risk in agricultural workers or their children
	Poisoning case reports suggest extrapyramidal syndrome, isolated bilateral vocal cord paralysis, pancreatitis and myocardial dysfunction following acute cholinergic crisis
	Many human volunteer data available with the critical effect of ChE inhibition
	Evidence of polyneuropathy following severe, life-threatening intoxications

Summary

	Value	Study	Safety factor
ADI	0–0.004 mg/kg bw	Human, 21-day oral dosing study	10
ARfD	0.1 mg/kg bw	Human, study of acute oral toxicity	10

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DICOFOL (addendum)

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Explanation

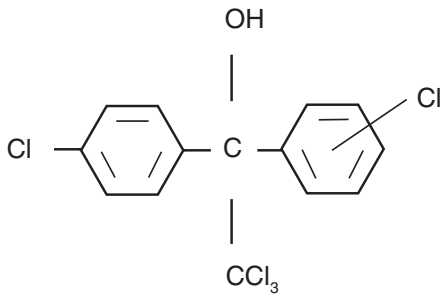
Dicofol is the International Organization for Standardization (ISO)–approved name of 2,2,2-trichloro-1,1-bis(4-chlorophenyl) ethanol (International Union of Pure and Applied Chemistry). Its Chemical Abstracts Service number is 115-32-2. Dicofol is structurally similar to dichlorodiphenyltrichloroethane (DDT). It is a non-systemic acaricide that acts by stimulating axonal transmission of nervous signals.

Dicofol was evaluated by the Joint FAO/WHO Meeting on Pesticide Residues in 1968 and in 1992, when an acceptable daily intake (ADI) of 0–0.002 mg/kg body weight (bw) was established. It was reviewed by the present Meeting within the periodic review programme of the Codex Committee on Pesticide Residues. Relevant parts of the most recent monograph have been incorporated into this toxicological evaluation. New studies on acute and short-term dermal toxicity, dermal hypersensitivity, skin and eye irritation, acute and subchronic neurotoxicity and reproductive toxicity, as well as supplementary studies on reproductive toxicity, carcinogenicity and mutagenicity, were provided and reviewed.

All pivotal studies with dicofol were certified as complying with good laboratory practice (GLP).

The chemical structure of dicofol is shown in Figure 1.

Figure 1. Chemical structure of dicofol



Evaluation for acceptable daily intake

Unless otherwise stated, studies evaluated in this monograph were performed by GLP-certified laboratories and complied with the relevant Organisation for Economic Co-operation and Development (OECD) and/or United States Environmental Protection Agency (USEPA) test guidelines.

Many of the studies reported on below that were previously evaluated by the Meeting and included in the previous monograph on dicofol (Annex 1, reference *67*) are reported here with no or only slight modifications (including expansion with more detailed information).

1. Biochemical aspects

1.1 Absorption, distribution and excretion

The disposition of a single oral dose of either [^{14}C]*p,p′*-dicofol, the active ingredient of Kelthane miticide (purity 96.88%), or [^{14}C]DDT (purity 95.68%) was studied in male and female Sprague-Dawley rats. Both compounds were uniformly radiolabelled in the chlorophenyl rings. Four rats of each sex received a 50 mg/kg bw single oral dose of [^{14}C]*p,p′*-dicofol or [^{14}C]DDT in corn oil. The treated animals were housed individually in metabolism cages. Excreta were collected twice daily for 3 days after administration of the ^{14}C-labelled compounds and then daily until termination for ^{14}C

Table 1. Recovery of ^{14}C after oral administration of [^{14}C]p,p'-dicofol or [^{14}C]DDT to rats[a]

Group	Animal no.	Sex	Compound	Termination time after dose (h)	% of ^{14}C dose recovered				
					Urine	Faeces	Wash	Tissues	Total
A-1	1	Male	*p,p'*-Dicofol	24	2.87	40.99	0.10	85.99	129.95
A-2	2	Male	*p,p'*-Dicofol	48	6.05	45.39	0.09	64.35	119.88
A-3	3	Male	*p,p'*-Dicofol	96	10.77	98.57	0.13	14.67	124.14
A-4	4	Male	*p,p'*-Dicofol	192	19.63	100.95	0.35	1.51	122.05
B-1	1	Female	*p,p'*-Dicofol	24	4.29	9.94	0.09	108.52	123.94
B-2	2	Female	*p,p'*-Dicofol	48	14.38	22.04	0.25	81.92	118.59
B-3	3	Female	*p,p'*-Dicofol	96	24.39	66.60	0.79	10.54	102.32
B-4	4	Female	*p,p'*-Dicofol	192	42.43	56.51	1.31	2.26	103.41
C-1	1	Male	DDT	24	0.94	51.10	0.13	44.16	96.33
C-2	2	Male	DDT	48	1.35	35.85	0.003	67.23	104.46
C-3	3	Male	DDT	96	4.35	77.01	0.14	23.34	104.84
C-4	4	Male	DDT	192	3.97	73.20	0.05	24.52	101.82
D-1	1	Female	DDT	24	1.28	13.54	0.19	96.58	111.59
D-2	2	Female	DDT	48	4.27	23.78	0.98	58.57	67.60
D-3	3	Female	DDT	96	6.86	43.06	0.31	47.66	97.89
D-4	4	Female	DDT	192	12.46	64.03	0.28	15.44	93.01

From Steigerwalt, Deckert & Longacre (1993)

[a] Each rat received a single oral dose of 50 mg/kg bw of the respective ^{14}C-labelled compound.

analysis. At 24, 48, 96 and 192 hours post-dosing, single male and female [^{14}C]*p,p'*-dicofol-treated rats and single male and female [^{14}C]DDT-treated rats were killed, and tissues were removed and analysed for total ^{14}C concentration. The recovery of ^{14}C after oral administration of [^{14}C]*p,p'*-dicofol and [^{14}C]DDT is shown in Table 1.

Results showed that urinary ^{14}C excretion was higher in both sexes after administration of [^{14}C]*p,p'*-dicofol than after administration of [^{14}C]DDT; females exhibited a higher ^{14}C excretion for each compound compared with males. Tissue ^{14}C residues were lower in [^{14}C]*p,p'*-dicofol-treated animals than in [^{14}C]DDT-treated animals, indicating almost complete elimination by 192 hours.

In general, maximum ^{14}C tissue levels were reached by 24 hours post-dosing after treatment with either ^{14}C-labelled compound and were similar in each tissue for each sex; ^{14}C tissue levels were generally higher in females than in males. Fat contained the greatest peak ^{14}C concentrations (282–978 µg/g); other tissues containing relatively high peak ^{14}C concentrations included adrenals (186–269 µg/g), gonads (12–95 µg/g) and caecum (54–96 µg/g). The ^{14}C label in tissues was eliminated 2- to 3-fold faster after [^{14}C]*p,p'*-dicofol treatment than after [^{14}C]DDT treatment. The mean half-life for elimination of ^{14}C label for all tissues studied is shown in Table 2.

The results indicated that ^{14}C label derived from a single oral dose of [^{14}C]*p,p'*-dicofol was distributed to the same tissues in approximately similar amounts in rats as ^{14}C label derived from a single oral dose of [^{14}C]DDT; however, [^{14}C]*p,p'*-dicofol-derived ^{14}C label was eliminated more rapidly and more completely than [^{14}C]DDT-derived ^{14}C label (Steigerwalt, Deckert & Longacre, 1993).

In an early study, the distribution and excretion of a single oral dose of 50 mg/kg bw [^{14}C]*o,p'*-dicofol or [^{14}C]*p,p'*-dicofol in corn oil (5 ml/kg bw) were studied in young adult female Clr:CD BR rats (n = 77). Urine, faeces, whole blood, fat, liver, adrenals and thyroids were collected at various

Table 2. Half-lives for the elimination of ^{14}C label from tissues of rats treated with [^{14}C]p,p′-dicofol or [^{14}C]DDT[a]

Test substance	Sex	Number of tissues	Half-life (h)[b]
[^{14}C]*p*,*p*′-Dicofol	Males	22	31.5 ± 9.9
[^{14}C]*p*,*p*′-Dicofol	Females	23	30.0 ± 9.5
[^{14}C]DDT	Males	15	94.4 ± 41.6[c]
[^{14}C]DDT	Females	21	54.9 ± 18.4[c]

From Steigerwalt, Deckert & Longacre (1993)

[a] Each rat received a single oral dose of 50 mg/kg bw of the respective test compound.

[b] Values represent half-life ± standard deviation.

[c] Significantly different ($P < 0.05$) from each of the other three groups.

Table 3. Overall recovery of ^{14}C label 10 days after dosing of female rats with a single oral dose of [^{14}C]o,p′-dicofol or [^{14}C]p,p′-dicofol

Group	[^{14}C]Dicofol isomer	% of dose				
		Urine[a]	Faeces	Cage wash	Tissues and carcass	Total recovery
A	*o*,*p*′-Dicofol	22	78	0.2	1	101
B	*p*,*p*′-Dicofol	20	61	1	22	104

From DiDonato, Steigerwalt & Longacre (1987)

[a] Includes ^{14}C label recovered from the urine funnel wash.

times after dosing for up to 10 days post-dosing and were analysed for ^{14}C label. The remaining carcass was also analysed for ^{14}C label.

The overall recovery of ^{14}C label from rats administered a single oral dose of [^{14}C]*o*,*p*′-dicofol and [^{14}C]*p*,*p*′-dicofol is shown in Table 3. The ^{14}C label was predominantly recovered in the faeces for both *o*,*p*′- and *p*,*p*′-dicofol (78% and 61%, respectively). A substantial amount of ^{14}C label was also recovered in the urine for both *o*,*p*′- and *p*,*p*′-dicofol; together with ^{14}C label recovered in the urine funnel wash, it amounted to 22% and 20%, respectively. The remaining ^{14}C label was recovered in the cage wash, tissues and remaining carcass. ^{14}C label recovered in the tissues and remaining carcass in the 10 days after dosing was considerably higher in rats administered *p*,*p*′-dicofol (22%) than in those administered *o*,*p*′-dicofol (1%). Following oral administration, most (over 90%) [^{14}C]*o*,*p*′-dicofol-derived ^{14}C label was eliminated in excreta within 2 days (and 100% eliminated in 10 days), compared with 40% of the [^{14}C]*p*,*p*′-dicofol-derived ^{14}C label (about 80% eliminated in 10 days).

Peak tissue concentrations were reached within 6 hours in most tissues and after 1–2 days in fat. Both isomers showed a high affinity for adipose tissue. At the time of peak concentrations, approximately 51% of *p*,*p*′-dicofol radiolabel and 26% of *o*,*p*′-dicofol radiolabel were in body fat (assuming fat is 7% of body weight). Tissue concentrations of both isomers were similar initially, but concentrations of *o*,*p*′-dicofol radiolabel declined more rapidly than those of *p*,*p*′-dicofol radiolabel. After 10 days, concentrations of *p*,*p*′-dicofol radiolabel were as follows: fat, 144 µg/g; adrenal gland, 30 µg/g; thyroid, 16 µg/g; liver, 6 µg/g; and whole blood, 1 µg/g. In comparison, *o*,*p*′-dicofol radiolabel concentrations were as follows: fat, 3 µg/g; adrenal gland and thyroid, 1 µg/g; blood, 0.6 µg/g; and liver, 0.5 µg/g. Elimination half-lives were estimated to be 1.5–4 days for *o*,*p*′-dicofol and 4–7 days for *p*,*p*′-dicofol. On the basis of this study, it is concluded that mobilization from fat is the rate-limiting step in the elimination of dicofol from the body (DiDonato, Steigerwalt & Longacre, 1987; reported in Annex 1, reference *67*).

The distribution of *p*,*p*′-dicofol to the bone marrow was studied in adult male and female Crl:CD BR mice. The study was conducted in support of a previous study (Sames & Doolittle, 1986).

Table 4. Recovery of [^{14}C]*p,p'*-dicofol (group A, 6-hour termination)[a]

Sex	% recovery							
	Urine + urine funnel wash	Faeces	Urine cage wash	Carcass	Bone marrow	Whole blood	Plasma[b]	Total
Male	0.03	0.01	0.00	93.42	0.000 27	0.002 98	0.000 28	93.46
Male	0.06	6.41	0.08	81.37	0.000 27	0.039 44	0.000 44	87.96
Male	0.03	0.53	0.04	77.71	0.000 32	0.000 29	0.000 30	78.31
Mean	**0.04**	**2.32**	**0.04**	**84.17**	**0.000 29**	**0.142 4**	**0.000 34**	**86.58**
SD	*0.02*	*3.55*	*0.04*	*8.22*	*0.000 03*	*0.218 7*	*0.000 09*	*7.67*
Female	0.05	0.00	0.01	125.94	0.000 26	0.025 98	0.000 33	126.03
Female	0.13	2.39	0.09	92.89	0.001 19	0.002 09	0.000 72	95.50
Female	0.16	0.03	0.25	103.72	0.000 35	0.033 54	0.000 52	104.19
Mean	**0.11**	**0.81**	**0.12**	**107.52**	**0.000 60**	**0.020 54**	**0.000 52**	**100.57**
SD	*0.06*	*1.37*	*0.12*	*16.85*	*0.000 51*	*0.016 42*	*0.000 20*	*15.73*

From Swenson & Hazelton (1994)
SD, standard deviation
[a] All animals received a single gavage dose (478 mg/kg bw) of [^{14}C]*p,p'*-dicofol at a constant volume of 10 ml/kg bw.
[b] Plasma values were not included in the total column, as plasma was accounted for in whole blood samples.

Table 5. Recovery of [^{14}C]*p,p'*-dicofol (group B, 24-hour termination)[a]

Sex	Urine + urine funnel wash	Faeces	Urine cage wash	Carcass	Bone marrow	Whole blood	Plasma[b]	Total
Male	0.40	27.00	1.20	56.17	0.002 24	0.016 36	0.002 98	84.79
Male	0.40	1.88	0.99	88.79	0.001 69	0.158 86	0.003 07	92.22
Male	0.37	14.64	0.33	68.63	0.000 95	0.005 93	0.002 45	83.98
Mean	**0.39**	**14.51**	**0.84**	**71.20**	**0.001 63**	**0.060 38**	**0.002 83**	**87.00**
SD	*0.02*	*12.56*	*0.45*	*16.46*	*0.000 65*	*0.085 44*	*0.000 34*	*4.54*
Female	1.15	17.99	3.68	54.87	0.002 27	0.089 70	0.001 38	77.78
Female	0.63	2.66	1.32	86.57	0.002 78	0.000 89	0.001 93	91.18
Female	0.75	20.58	3.56	72.87	0.002 75	0.131 44	0.003 03	97.89
Mean	**0.84**	**13.74**	**2.85**	**71.44**	**0.002 60**	**0.074 00**	**0.002 11**	**88.95**
SD	*0.27*	*9.69*	*1.33*	*15.90*	*0.000 29*	*0.667 0*	*0.000 84*	*20.24*

From Swenson & Hazelton (1994)
SD, standard deviation
[a] All animals received a single gavage dose (478 mg/kg bw) of [^{14}C]*p,p'*-dicofol at a constant volume of 10 ml/kg bw.
[b] Plasma values were not included in the total column, as plasma was accounted for in whole blood samples.

A single gavage dose (478 mg/kg bw, expressed as active ingredient [a.i.]) of [^{14}C]*p,p'*-dicofol was administered to three groups of rats (three of each sex per group). Rats were killed at 6 (group A), 24 (group B) and 48 (group C) hours after administration of the test material, and bone marrow tissue samples were collected and analysed for ^{14}C content. Urine, urine funnel wash and faecal samples were collected continuously.

The distribution of [^{14}C]*p,p'*-dicofol is shown in Tables 4, 5 and 6. Total recovery of administered ^{14}C label ranged from 78% to 126% for individual animals. At all termination times, ^{14}C label derived from [^{14}C]*p,p'*-dicofol was present in the bone marrow of both male and female rats. The distribution of ^{14}C label to the bone marrow is shown in Tables 7, 8 and 9. Mean concentrations (male/female values) observed at 6, 24 and 48 hours were 6.1/10.6, 32.1/52.7 and 25.2/70.8 µg equivalents

Table 6. Recovery of [^{14}C]*p,p'*-dicofol (group C, 48-hour termination)[a]

Sex	Urine + urine funnel wash	Faeces	Urine cage wash	Carcass	Bone marrow	Whole blood	Plasma[b]	Total
Male	2.79	9.67	0.28	87.50	0.001 92	0.000 02	0.003 92	100.17
Male	1.89	31.31	0.31	54.63	0.000 92	0.216 61	0.002 81	88.36
Male	1.53	31.85	0.43	61.61	0.000 95	0.005 24	0.003 09	95.43
Mean	**2.05**	**24.28**	**0.34**	**67.91**	**0.001 26**	**0.073 96**	**0.003 27**	**94.65**
SD	*0.61*	*12.68*	*0.08*	*17.32*	*0.000 57*	*0.123 57*	*0.000 58*	*5.94*
Female	5.13	15.36	0.91	64.90	0.002 82	0.222 63	0.002 66	86.53
Female	3.06	16.88	0.86	78.57	0.004 12	0.003 24	0.004 03	99.38
Female	3.67	13.01	1.20	80.17	0.001 26	0.175 41	0.003 34	98.23
Mean	**3.95**	**15.08**	**0.99**	**74.55**	**0.002 73**	**0.133 76**	**0.003 34**	**94.71**
SD	*1.06*	*1.95*	*0.18*	*8.39*	*0.001 43*	*0.115 45*	*0.000 69*	*7.11*

From Swenson & Hazelton (1994)
SD, standard deviation
[a] All animals received a single gavage dose (478 mg/kg bw) of [^{14}C]*p,p'*-dicofol at a constant volume of 10 ml/kg bw.
[b] Plasma values were not included in the total column, as plasma was accounted for in whole blood samples.

Table 7. Distribution of ^{14}C label to the bone marrow in rats (group A)[a]

Sex	Distribution to the bone marrow	
	Concentration (ppm)[b]	% of dose[c]
Male	6.499	0.000 27
Male	6.556	0.000 27
Male	5.322	0.000 32
Mean	**6.126**	**0.000 29**
SD	*0.697*	*0.000 03*
Female	5.242	0.000 26
Female	18.477	0.001 19
Female	8.122	0.000 35
Mean	**10.614**	**0.000 60**
SD	*6.960*	*0.000 51*

From Swenson & Hazelton (1994)
SD, standard deviation
[a] Rats were dosed with [^{14}C]*p,p'*-dicofol (478 mg/kg bw). At 6 hours, rats were killed and bone marrow tissue was collected. The individual value for each animal represents the mean of two bone marrow samples per animal.
[b] Values are expressed as parts per million (µg equivalents of [^{14}C]*p,p'*-dicofol per gram of bone marrow sample).
[c] Values are expressed as percentage of administered radioactive dose in recovered bone marrow samples (approximately 20–40 mg per sample) of the rat femur.

of [^{14}C]*p,p'*-dicofol per gram of bone marrow, respectively. At 6 hours (group A), 84–108% of the ^{14}C label was recovered in the carcass, whereas only 1–2% was recovered in the excreta (faeces, urine, urine funnel wash and urine cage wash), bone marrow and whole blood, combined. After 24 hours (group B), 71% of the ^{14}C label was recovered in the carcass, 14–15% in the faeces and 1–4% in the remaining excreta, bone marrow and whole blood, combined. After 48 hours (group C), 68–75% of the ^{14}C label was recovered in the carcass, 15–24% in the faeces and 2–5% in the remaining excreta, bone marrow and whole blood, combined.

These findings demonstrate that *p,p'*-dicofol distributes to bone marrow tissue following a single oral dose (478 mg/kg bw) of the test material to adult male and female rats. This information

Table 8. Distribution of ^{14}C label to the bone marrow in rats (group B)[a]

Sex	Distribution to the bone marrow	
	Concentration (ppm)[b]	% of dose[c]
Male	39.432	0.002 24
Male	35.112	0.001 69
Male	21.641	0.000 95
Mean	**32.062**	**0.001 63**
SD	*9.279*	*0.000 65*
Female	54.437	0.002 27
Female	57.100	0.002 78
Female	46.579	0.002 75
Mean	**52.705**	**0.002 60**
SD	*5.470*	*0.000 29*

From Swenson & Hazelton (1994)
SD, standard deviation
[a] Rats were dosed with [^{14}C]*p,p′*-dicofol (478 mg/kg bw). At 24 hours, rats were killed and bone marrow tissue was collected. The individual value for each animal represents the mean of two bone marrow samples per animal.
[b] Values are expressed as parts per million (µg equivalents of [^{14}C]*p,p′*-dicofol per gram of bone marrow sample).
[c] Values are expressed as percentage of administered radioactive dose in recovered bone marrow samples (approximately 20–40 mg per sample) of the rat femur.

Table 9. Distribution of ^{14}C label to the bone marrow in rats (group C)[a]

Sex	Distribution to the bone marrow	
	Concentration (ppm)[b]	% of dose[c]
Male	37.859	0.001 92
Male	17.591	0.000 92
Male	20.196	0.000 95
Mean	**25.215**	**0.001 92**
SD	*11.027*	*0.000 57*
Female	72.836	0.002 82
Female	104.203	0.004 12
Female	35.368	0.001 26[d]
Mean	**70.802**	**0.002 73**
SD	*34.463*	*0.001 43*

From Swenson & Hazelton (1994)
SD, standard deviation
[a] Rats were dosed with [^{14}C]*p,p′*-dicofol (478 mg/kg bw). At 48 hours, rats were killed and bone marrow tissue was collected. The individual value for each animal represents the mean of two bone marrow samples per animal.
[b] Values are expressed as parts per million (µg equivalents of [^{14}C]*p,p′*-dicofol per gram of bone marrow sample).
[c] Values are expressed as percentage of administered radioactive dose in recovered bone marrow samples (approximately 20–40 mg per sample) of the rat femur.
[d] This value represents only one of two bone marrow sample for this animal. The second sample was excluded, as it was considered atypical when compared with all other values in the group.

provides support for a previous in vivo cytogenetic study (Sames & Doolittle, 1986) conducted with dicofol (Kelthane technical), in that the test material was shown to reach the primary tissue site for this assay (Swenson & Hazelton, 1994).

The disposition of dicofol and DDT was compared following multiple oral doses using female Sprague-Dawley rats (groups of 1–2 rats) given daily doses of 0.5 mg [^{14}C]*p,p′*-dicofol or [^{14}C]*p,p′*-DDT (uniformly ring labelled) per kilogram body weight for 16 consecutive days. Blood, urine, faeces and tissues were collected during treatment and over 16 days after exposure. As in the single-dose comparison study (Steigerwalt, Deckert & Longacre, 1993), DDT and dicofol radiolabel showed qualitatively similar distribution and elimination patterns, but DDT was more persistent. Dicofol radiolabel was excreted approximately twice as fast as DDT radiolabel. Approximately 75% of the dicofol dose was excreted within 16 days, compared with 40% of the DDT dose. Both were eliminated mainly in the faeces. Concentrations in tissues, such as fat, liver and adrenal glands, were comparable during treatment, but dicofol radiolabel was eliminated from these tissues more rapidly. These results are comparable to those obtained after a single oral dose of [^{14}C]*p,p′*-dicofol or [^{14}C]-DDT in rats. Fat concentrations of DDT radiolabel increased and peaked post-exposure, whereas dicofol radiolabel began declining when exposure ceased. After 16 days, the concentration of DDT radiolabel in fat was twice that of dicofol radiolabel (38 versus 13 µg/g). Elimination half-lives were estimated to be 6–14 days for dicofol and 7–24 days for DDT (Steigerwalt, Deckert & Longacre, 1984b; reported in Annex 1, reference *67*).

1.2 Biotransformation

The metabolism of dicofol was studied in male and female Sprague-Dawley rats following administration of a single oral dose of 50 mg/kg bw of [^{14}C]*p,p′*-dicofol (purity 98.0%) (uniformly labelled ring). Blood, urine, faeces and tissues (liver, kidney, fat) were collected over 7 days. The distribution of dicofol and its metabolites was determined at 0–48 and 48–168 hours.

In the faeces, most of the extracted radiolabel was present as FW-152. As the major dicofol metabolite, it represented 36.6% ± 25.7% and 51.0% ± 25.0% of the excreted ^{14}C in 0- to 48-hour male and female faecal samples, respectively, and 16.6% ± 20.4% and 27.1% ± 11.3% of the ^{14}C in 48- to 168-hour male and female faecal samples, respectively. Male faecal samples showed higher concentrations of dichlorobenzhydrol (DCBH) (29.54% ± 20.6% to 34.5% ± 20.3%) than did female samples (5.87% ± 4.68% to 12.9% ± 3.23%). In contrast, females excreted more hydroxyl dichlorobenzophenone (OH-DCBP) through the faeces (10.1% ± 9.81% to 22.8% ± 6.53%) compared with males (5.07% ± 2.68% to 9.91% ± 3.86%) (Table 10).

The major identified metabolites in male urine extracts were OH-DCBP/DCBH (24.4% ± 6.4% to 30.8% ± 9.66% of extracted ^{14}C) and dichlorobenzilic acid (DCBA)-glycine (13.9% ± 7.81% to 10.5% ± 5.96%). Female extracts contained a metabolite profile similar to that of males (Table 11).

Concentrations of ^{14}C residues in the tissues of male and female rats measured 0–168 hours post-dosing were highest in adipose tissue. An average of 82.9% ± 9.58% for male and 89.2% ± 4.32% for female rat adipose tissue was identified as dicofol. In addition to dicofol, male and female adipose tissue contained FW-152 and dichlorobenzophenone (DCBP) (Table 12).

Liver extracts contained highly polar metabolites, although FW-152 constituted an average of 72.8% ± 8.19% and 77.7% ± 4.26% of the petroleum ether partition from male and female samples, respectively. Analysis of the ethyl acetate liver partition, which contained 15% of the total liver ^{14}C, showed a number of polar dicofol metabolites, all comprising less than 6% of the extracted ^{14}C, with the exception of FW-152, which comprised 16.6% ± 1.13% and 52.9% of the male and female ^{14}C in the ethyl acetate partition, respectively (Table 13).

Blood plasma extracts contained highly polar metabolites. Although small amounts of the dicofol metabolites DCBH, DCBP, FW-152, DCBA and DCBA-glycine were detected, each comprising 10% of the total extracted ^{14}C, 30–77% of the extracted ^{14}C was more polar than the most polar dicofol metabolite, chlorohippuric acid (CHA), and could not be identified (Table 14).

Table 10. Distribution of metabolites in faecal extracts from male and female rats after a single oral dose of [^{14}C]p,p'-dicofol

	% of administered radioactivity (mean ± standard deviation)			
	Males		Females	
	0–48 h[a]	48–168 h[b]	0–48 h[a]	48–168 h[c]
Average % of total ^{14}C dose recovered	44.35 ± 3.69	61.70 ± 12.9	31.96 ± 9.25	31.95 ± 5.64
Average % of ^{14}C in methanol extract	92.70 ± 18.8	93.10 ± 16.8	96.80 ± 18.7	86.30 ± 17.0
Average % recovery from Sep-Pak purification	95.80 ± 7.49	91.80 ± 9.71	86.60 ± 8.87	86.00 ± 16.6
Average % of ^{14}C in Sep-Pak eluent				
- dicofol	4.87 ± 7.23[d]	2.07 ± 0.90[d]	4.74 ± 4.63	2.62 ± 1.44[e]
- DCBP	2.97 ± 0.72[f]	2.13 ± 0.62	2.89 ± 1.26	2.54 ± 1.49[f]
- FW-152	36.60 ± 25.7	16.60 ± 20.4	51.10 ± 25.0	27.10 ± 11.3
- DCBH	29.54 ± 20.6	34.50 ± 20.3	5.87 ± 4.68	12.9 ± 3.23
- OH-DCBP	5.07 ± 2.68[g]	9.91 ± 3.86	10.1 ± 9.81	22.8 ± 6.53
- OH-DCBH/DCBA-glycine	5.91 ± 6.03	10.40 ± 7.61	4.08 ± 3.27	6.70 ± 3.39
- "DDE" zone	2.63 ± 2.16[h]	0.88 ± 0.38	1.01 ± 0.64	1.27 ± 1.26
- unidentified (includes baseline + 3–10 minor zones)	12.8 ± 9.50	21.10 ± 17.9	19.1 ± 17.6	23.6 ± 13.9

From Tillman & Mazza (1986)
DCBA, dichlorobenzilic acid; DCBP, dichlorobenzophenone; DCBH, dichlorobenzhydrol; DDD, dichlorodiphenyldichloroethane; DDE, dichlorodiphenyldichloroethene; OH-DCBP, hydroxyl dichlorobenzophenone; OH-DCBH, hydroxyl dichlorobenzhydrol
[a] Total of 10 samples from five animals chosen as representative samples.
[b] Total of eight samples from three animals chosen as representative samples.
[c] Total of nine samples from nine animals chosen as representative samples.
[d] Some DCBP and DDD residues are included in these data.
[e] Some DCBP residues are included in these data.
[f] Some dicofol residues are included in these data.
[g] Some DCBH residues are included in these data (no separation from OH-DCBP on thin-layer chromatography).
[h] Data are from a zone that co-chromatographed with DDE but was later shown not to be DDE.

Table 11. Distribution of metabolites in urine extracts from male and female rats after a single oral dose of [^{14}C]p,p'-dicofol

	% of administered radioactivity (mean ± standard deviation)			
	Males		Females	
	0–48 h[a]	48–168 h[b]	0–48 h[a]	48–168 h[b]
% of total ^{14}C dose recovered	5.59 ± 2.19	16.33 ± 9.72	5.72 ± 1.46	19.40 ± 12.50
% of ^{14}C in ethyl acetate extract	80.3 ± 13.0	81.9 ± 13.8	98.9 ± 32.8	95.0 ± 26.6
% recovery of ^{14}C extracts as:				
- DCBP	1.62 ± 0.92	1.66 ± 0.30	1.21 ± 0.46	1.28 ± 0.62
- OH-DCBP/DCBH[c]	24.4 ± 6.4	30.8 ± 9.66	23.3 ± 5.94	22.1 ± 9.04
- OH-DCBH/CBA[c]	5.34 ± 2.14	5.60 ± 2.61	3.46 ± 1.88	2.46 ± 1.50
- DCBA	4.00 ± 6.01	3.78 ± 4.63	2.26 ± 0.96	1.37 ± 0.81
- DCBA-glycine/CHA[c]	24.7	7.32 ± 3.69	22.1 ± 9.47	10.0 ± 6.24
- CHA[d]	5.11 ± 3.22	6.43 ± 2.60	6.05 ± 1.91	5.65 ± 0.07
- DCBA-glycine[d]	13.9 ± 7.81	10.5 ± 5.96	19.75 ± 1.34	17.7 ± 3.96
- unknown metabolite	12.2 ± 10.5	17.2 ± 15.9	9.83 ± 4.98	17.2 ± 11.8
- unidentified (baseline + 5–10 minor zones)	33.2 ± 13.8	29.4 ± 12.8	38.1 ± 10.3	40.2 ± 15.6

From Tillman & Mazza (1986)
CBA, chlorobenzoic acid; CHA, chlorohippuric acid; DCBA, dichlorobenzilic acid; DCBP, dichlorobenzophenone; DCBH, dichlorobenzhydrol; OH-DCBP, hydroxyl dichlorobenzophenone; OH-DCBH, hydroxyl dichlorobenzhydrol
[a] Total of nine samples analysed from five animals chosen as representative samples.
[b] Total of nine samples analysed from three animals chosen as representative samples.
[c] These metabolites were inseparable on most of the thin-layer chromatographic plates; percentage reported represents the sum of both compounds.
[d] These data were generated from the analyses in which CHA and DCBA-glycine were separable.

Table 12. Distribution of metabolites in fat extracts from male and female rats after a single oral dose of [^{14}C]p,p'-dicofol

	% of administered radioactivity (mean ± standard deviation)			
	Males[a]		Females[b]	
	0–48 h	48–168 h	0–48 h	48–168 h
Average total % of ^{14}C dose recovered	34.2 ± 12.2	3.62 ± 4.59	83.3 ± 23.0	22.6 ± 14.9
% of ^{14}C in petroleum ether extract	143 ± 66.9	94.6	93.4 ± 7.50	101.7 ± 17.5
% of ^{14}C extract as:				
- dicofol	82.9 ± 9.58		89.2 ± 4.32	
- DCBP	2.67 ± 0.06		2.80 ± 0.68	
- FW-152	7.77 ± 2.74		3.83 ± 2.46	
- DCBH	0.73 ± 0.75		0.18 ± 0.15	
- OH-DCBP	0.43 ± 0.35		0.30 ± 0.29	
- OH-DCBH	1.37 ± 1.95		0.93 ± 1.09	
- "DDE" zone[c]	0.63 ± 1.01		0.33 ± 0.59	
- unidentified	3.53 ± 3.46		2.45 ± 1.42	

From Tillman & Mazza (1986)
DCBP, dichlorobenzophenone; DCBH, dichlorobenzhydrol; DDE, dichlorodiphenyldichloroethene; OH-DCBP, hydroxyl dichlorobenzophenone; OH-DCBH, hydroxyl dichlorobenzhydrol
[a] Total of three samples analysed (two from 0- to 48-hour and one from 48- to 168-hour group).
[b] Total of four samples analysed (two from each group).
[c] Data were generated from a zone that co-chromatographed with DDE but was shown not to be DDE.

Table 13. Distribution of metabolites in liver extracts from male and female rats after a single oral dose of [^{14}C]p,p'-dicofol

	% of administered radioactivity (mean ± standard deviation)			
	Males		Females	
	0–48 h[a]	48–168 h[b]	0–48 h[a]	48–168 h[b]
Average total % of ^{14}C dose recovered	2.83 ± 0.94	0.28 ± 0.11	2.03 ± 0.30	0.69 ± 0.28
Average % of ^{14}C in methanol extract	86.1 ± 27.8	90.8	73.6 ± 25.1	60.9 ± 16.8
Average % of ^{14}C in petroleum ether extract	83.6 ± 7.85	88.4	90.5 ± 0.07	81.3 ± 9.26
Average % of ^{14}C petroleum ether extracts as:				
- dicofol	2.97 ± 1.00[c]		3.70 ± 2.30[c]	
- DCBP	2.00 ± 0.57		2.27 ± 1.40	
- FW-152	72.8 ± 8.19[d]		77.7 ± 4.26	
- DCBH	6.45 ± 2.47		4.43 ± 0.61	
- OH-DCBP	2.37 ± 0.96		4.33 ± 2.48	
- OH-DCBH	4.10 ± 1.08		1.45 ± 0.73	
- DCBA-glycine[e]	3.05 ± 3.32		1.55 ± 1.91	
- "DDE" zone	0.77 ± 0.55		0.43 ± 0.29	
- unidentified	5.47 ± 3.50		4.68 ± 1.56	
Average % ^{14}C in ethyl acetate partition	13.5 ± 3.89	18.9	12.5 ± 2.19	16.9 ± 1.98
Average % ^{14}C in ethyl acetate partition as:				

Table 13 (continued)

	% of administered radioactivity (mean ± standard deviation)			
	Males		Females	
	0–48 h[a]	48–168 h[b]	0–48 h[a]	48–168 h[b]
- dicofol	0.95 ± 0.35[c]		3.80[c]	
- DCBP	0.60 ± 0.30		1.23 ± 0.73	
- FW-152	21.9 ± 10.2[f]		48.2 ± 11.9[f]	
	16.6 ± 1.13[g]		52.9[g]	
- DCBH	2.85 ± 0.07		3.00 ± 1.16	
- OH-DCBP	2.0 ± 0.0[f]		3.0 ± 1.82[f]	
	4.9 ± 4.10[g]		3.10[g]	
- DCBA-glycine	5.47 ± 5.67		3.78 ± 6.78	
- CBA/OH-DCBH[h]	5.37 ± 5.85		1.57 ± 0.50	
- DCBA	2.77 ± 0.70		3.85 ± 1.58	
- CHA	3.60 ± 3.22		2.48 ± 2.70	
- unknown	24.8 ± 10.3		13.5 ± 5.91	
- unidentified (baseline + 4–11 minor bands)	42.0 ± 12.0		26.6 ± 7.73	

From Tillman & Mazza (1986)

CBA, chlorobenzoic acid; CHA, chlorohippuric acid; DCBA, dichlorobenzilic acid; DCBP, dichlorobenzophenone; DCBH, dichlorobenzhydrol; DDE, dichlorodiphenyldichloroethene; OH-DCBP, hydroxyl dichlorobenzophenone; OH-DCBH, hydroxyl dichlorobenzhydrol

[a] Total of three samples analysed (two from 0- to 48-hour group and one from 48- to 168-hour group) as representative samples.

[b] Total of four samples analysed (two from each group) as representative samples.

[c] DCBP residues are included in data (inseparable from dicofol on thin-layer chromatography).

[d] One set of data includes DCBH residues (inseparable from FW-152 on thin-layer chromatography).

[e] Data are from a zone that co-chromatographed with DDE but was shown not to be DDE.

[f] Dicofol residues included in data (inseparable from FW-152 on thin-layer chromatography).

[g] These data were generated from separate analyses under less polar thin-layer chromatography conditions, which separated FW-152 and dicofol or DCBH and OH-DCBP.

[h] CBA and OH-DCBH residues are reported together, as they were inseparable under the thin-layer chromatography conditions used.

In this study, no significant amounts of dichlorodiphenyldichloroethene (DDE) were detected in the excreta or tissue samples. Small amounts of material in faeces co-chromatographed with DDE. Additional analyses by high-performance liquid chromatography (HPLC) determined that 0.27% of the extracted radiolabel in faeces was actually DDE. Additional analyses of fat and liver detected small amounts of DDE in one fat sample (0.2% of extract, 0.34 µg/g tissue concentration) and two liver samples (0.25–0.34% of extract, 0.018–0.29 µg/g tissue concentration). The ^{14}C dosing solution was reported to contain 0.01% DDE (Tillman & Mazza, 1986; reported in Annex 1, reference *67*).

The proposed metabolic scheme for dicofol in rats is shown in Figure 2.

2. Toxicological studies

2.1 Acute toxicity

The acute toxicity of technical dicofol is summarized in Table 15. Common signs of toxicity include decreased spontaneous motor activity, ataxia, passiveness, somnolence, prostration and occasionally tremors. In cats, dicofol given intravenously had no convulsive activity but produced

Table 14. Distribution of metabolites in blood plasma from male and female rats after a single oral dose of [^{14}C]p,p'-dicofol

	% of administered radioactivity (mean ± standard deviation)			
	Males[a]		Females[a]	
	0–48 h	48–168 h	0–48 h	48–168 h
Average total % of ^{14}C dose recovered	0.77 ± 0.38	0.11 ± 0.04	0.71 ± 0.29	0.29 ± 0.13
% of ^{14}C in extract	75.1 ± 2.26	33.8 ± 8.77	75.1 ± 18.9	55.9 ± 12.4
% of ^{14}C extract as:				
- DCBP/dicofol/FW-152[b]	11.8 ± 9.55	7.50 ± 1.41	14.3 ± 1.06	18.3 ± 0.35
- DCBH	4.50 ± 3.82[c]	1.55 ± 0.35	3.55 ± 0.21	2.45 ± 0.92
- OH-DCBP	2.80	1.95 ± 0.35	7.50 ± 0.99	3.55 ± 1.48
- OH-DCBH/CHA[d]	4.55 ± 0.64	3.80 ± 2.69	4.45 ± 1.78	3.00 ± 0.85
- DCBA	7.20 ± 5.52[e]	5.30 ± 0.57	0.75 ± 0.35	0.75 ± 0.21
- DCBA-glycine	9.20	4.65 ± 2.19	2.45 ± 0.49	2.25 ± 1.63
- unknown	31.9	4.4	12.6 ± 1.70	9.90
- unidentified (includes baseline + 4–6 minor zones)	29.4 ± 17.7	77.3 ± 8.3	54.5 ± 5.44	64.8 ± 7.35

From Tillman & Mazza (1986)

CHA, chlorohippuric acid; DCBA, dichlorobenzilic acid; DCBP, dichlorobenzophenone; DCBH, dichlorobenzhydrol; OH-DCBP, hydroxyl dichlorobenzophenone; OH-DCBH, hydroxyl dichlorobenzhydrol

[a] Total of four samples analysed, two from each group.

[b] These data represent total residues of DCBP, dicofol and FW-152 (inseparable by thin-layer chromatography).

[c] Some OH-DCBP residues are included (inseparable from DCBH by thin-layer chromatography).

[d] These metabolites were inseparable under the conditions used.

[e] Includes residues of unidentified metabolites (inseparable from DCBA).

cardiovascular effects consisting of prolonged arrhythmia and hypertension at sublethal doses and ventricular fibrillation at a lethal dose.

(a) Oral administration

Rats

A single dose of 0, 10, 15 or 75 mg/kg bw (expressed as active ingredient) at a constant volume of 5 ml/kg bw of dicofol (Kelthane technical miticide; purity 95.2%) was given to 132 Crl:CD BR rats by gavage. Five rats of each sex per group were euthanized and necropsied on post-dosing day 2, day 6 and day 14, respectively. Samples of the dosing suspensions containing dicofol at all dose levels were analysed for proximity to target concentration. The average proximity to target of the suspensions ranged from 94% to 101%.

Rats were observed approximately 2–4 hours after dosing and then daily thereafter for 14 days for mortality and signs of ill-health or reaction to treatment. Body weights were determined 2 days prior to dosing and on days 0 (i.e. fasted body weight), 1, 5 and 13. Feed consumption was also measured for days 1, 2–5 and 6–13. On day 2, day 6 and day 14, respectively, five rats of each sex per group were anaesthetized via carbon dioxide inhalation, and blood samples were collected from the abdominal aorta for clinical chemistry, haematology, white blood cell differential counts and determination of corticosterone and thyroid hormone levels (thyroid stimulating hormone [TSH], triiodothyronine [T_3] and thyroxine [T_4]). After blood samples were collected, rats were euthanized by exsanguination via the abdominal aorta, and complete gross examinations were performed. The liver was removed from each animal and immediately chilled in ice-cold saline solution. After chilling, the liver was removed from the saline solution, blotted dry and weighed. Appropriate sections for

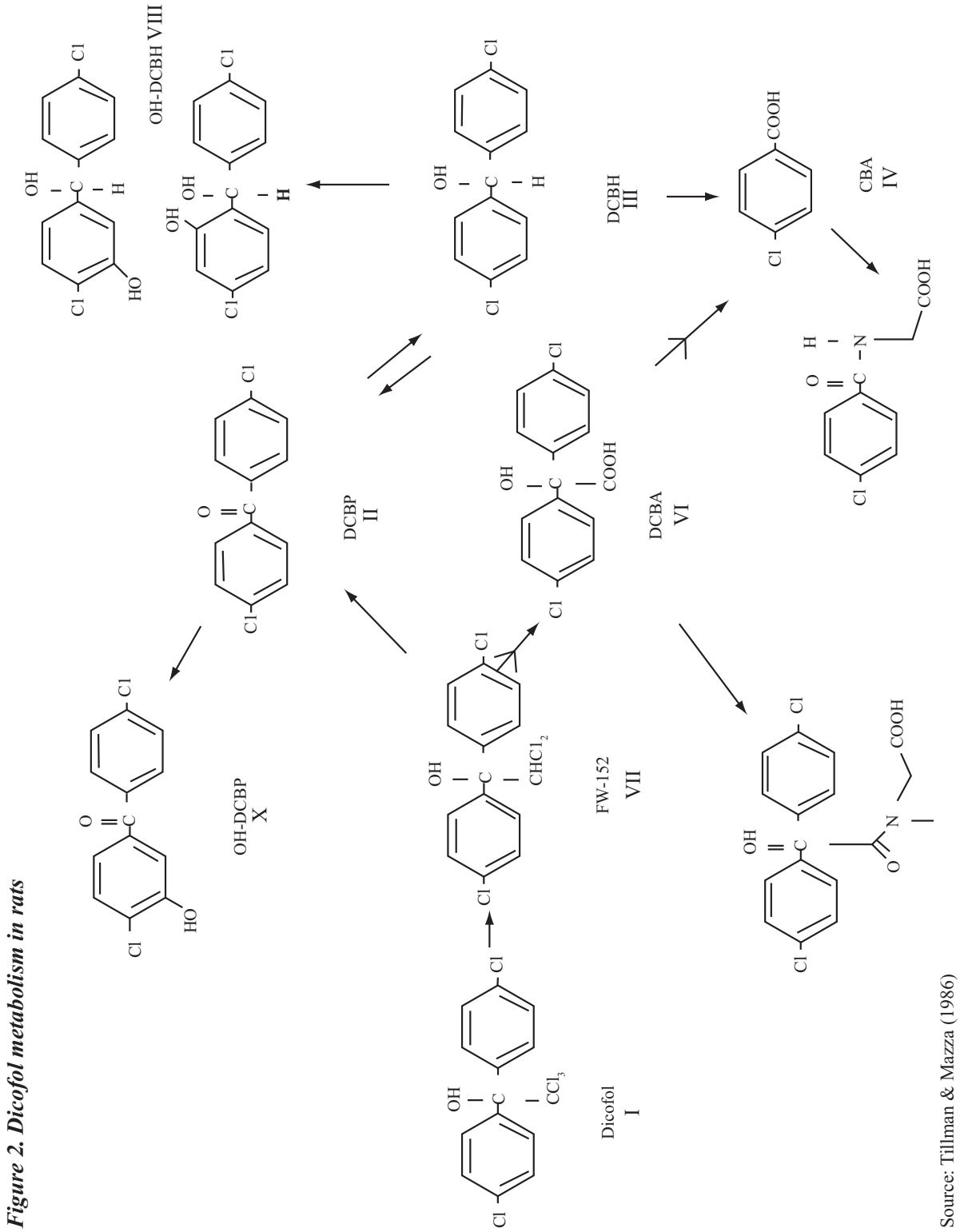


Figure 2. Dicofol metabolism in rats

Source: Tillman & Mazza (1986)

Table 15. Acute toxicity of dicofol

Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	LC_{50} (mg/l)	Purity (%)	Reference
Mouse	CRJ:CD-1 (ICR)	M	Oral	669	—	Unspecified	Onishi (1989)
		F		675			
Rat	CR:CD	M	Oral	595	—	94–96	Kryzwicki & Bonin (1985a)
		F	Oral	578			
Rat	?	M	Oral	809	—	80–85	Smith et al. (1959)
		F		684			
Rat	Wistar	M	Oral	1495[a]	—	80–85	Brown, Hughes & Viriyanondha (1969)
Rat	CR:CD BR	M/F	Dermal (24 h exposure)	> 5000	—	94–96	Ferguson, Craig & Eberly (2000a)
Rat	CR:CD	M/F	Dermal (24 h exposure)	> 5000	—	94–96	Krzywicki & Bonin, (1985b)
Rat	Wistar	M/F	Intraperitoneal	1115	—	Unspecified	De Groot (1974)
Rat	Wistar	M	Intraperitoneal	1150[a]	—	80–85	Brown, Hughes & Viriyanondha (1969)
Rat	Crl:CDBR	M/F	Inhalation (4 h exposure)	—	> 5.0	Unspecified	Fisher & Hagan (1987)
Rabbit	?	M	Oral	1810	—	80–85	Smith et al. (1959)
Rabbit	New Zealand White	F	Dermal (24 h exposure)	> 2500	—	95.2	Krzywicki & Bonin (1985b)
Cat	?	M	Intravenous	> 20	—	Unspecified	Joy (1976)
Dog	?	M/F	Oral	> 4000	—	80–85	Smith et al. (1959)

F, female; LC_{50}, median lethal concentration; LD_{50}, median lethal dose; M, male
[a] The observation period was 7 days only.

histopathological evaluation were taken, and the remaining liver sample was frozen for quantitative assessment of liver enzyme induction. The following tissues and organs were saved in 10% neutral buffered formalin: adrenals, heart, kidney, liver, lungs, ovaries, pituitary, spleen, testes, thyroid and any gross lesions. Organ weights were obtained for adrenals, kidney, liver, ovaries, pituitary, testes and thyroid. Animals scheduled for necroscopy on days 2, 6 and 14 also had their fasted body weights recorded just prior to blood collection. Histopathological evaluations were performed on all tissues collected from animals in the high-dose and control groups at each time point.

There were no mortalities or clinical signs of toxicity noted in any animal at any dose level at any time point.

There were no treatment-related effects on body weight in males or females at any dose level at any time point.

Treatment-related statistically significant decreases in average daily feed consumption (by 21–35% in males and by 41–48% in females for day 1 only) were noted in high-dose (75 mg/kg bw) rats.

There were no treatment-related effects on any haematology parameters, white blood cell differential parameters, clinical chemistry parameters, corticosterone or thyroid hormone levels (TSH, T_3 and T_4) in either sex at any time point.

There were no treatment-related effects on liver enzyme induction parameters in either sex at any time point in the low-dose group (10 mg/kg bw).

Statistically significant changes in 4-aminopyrine *N*-demethylation and cytochrome P450 were observed in mid-dose (15 mg/kg bw) males necropsied on day 2. These statistically significant findings were transient, in that no changes in these parameters were seen on day 6 in these dose groups. The transient effects on day 2 were not considered adverse, as they were not associated with changes in liver weight or with corresponding histopathological findings of the liver on day 2 or 6.

Treatment-related increases in 4-aminopyrine *N*-demethylation and cytochrome P450 content were observed in high-dose (75 mg/kg bw) males and females on days 2 and 6. Treatment-related increases in cytochrome b_5 content were also noted in high-dose (75 mg/kg bw) males and females on day 6. There were no treatment-related changes in cytochrome P450–related parameters at the high dose (75 mg/kg bw) for males or females on day 14.

There were no treatment-related effects on adrenal, kidney, ovary, pituitary, testes or thyroid weight. Treatment-related statistically significant increases in absolute (12%) and relative (14%) liver weights were noted in high-dose (75 mg/kg bw) males at day 2. These same parameters were also increased in high-dose females (6% and 10%, respectively) at day 2; however, these increases were not statistically significant. At day 6, treatment-related statistically significant increases in relative liver weights were observed in both sexes at the high dose (75 mg/kg bw) (8% in males and 12% in females). There were no treatment-related effects on liver weights in either sex at the high dose (75 mg/kg bw) at day 14 or in either sex at the middle (15 mg/kg bw) or low (10 mg/kg bw) dose at any time point.

There were no toxicologically significant gross findings in either sex at any time point.

A treatment-related microscopic change occurred in the adrenal cortex of three out of five high-dose (75 mg/kg bw) female rats on day 2. This change consisted of a mild or moderate enlargement (hypertrophy) of the cells of the zona fasciculata. A similar change was not observed in the male rats on day 2 or in the male or female rats on day 6 or day 14.

Transient increases in liver enzyme induction, as measured by 4-aminopyrine *N*-demethylation and cytochrome P450 content, were not considered adverse, toxicologically significant effects. Treatment-related increases in absolute and relative liver weights and liver enzyme induction parameters (i.e. 4-aminopyrine *N*-demethylation, cytochrome P450 content and cytochrome b_5 content) were noted at 75 mg/kg bw. On the basis of these data, the no-observed-adverse-effect level (NOAEL) was 15 mg/kg bw (expressed as active ingredient), based on decreased feed intake and hypertrophy of the adrenal zona fasciculata at 75 mg/kg bw (Parno, Anderson & Donofiro, 2000).

(b) Dermal application

Rats

Six male and female rats (Crl:CD BR) were exposed dermally to *p*,*p*′-dicofol (active ingredient in Kelthane technical miticide; purity 95.2%) at 5000 mg/kg bw applied to approximately 10% of the shaved body surface area. The test substance was maintained in contact with the skin for 24 hours using an occlusive dressing. The rats were observed for signs of ill-health or reaction to treatment at approximately 1, 2 and 4 hours after dosing and once daily thereafter for 14 days. Body weights were recorded on day 0 (prior to dosing) and on days 7 and 14. All rats were subjected to a postmortem examination at termination.

No mortalities or clinical signs of toxicity were noted in males. One female was found dead at day 8. Scant/no faeces, emaciation, hunched posture, passiveness and ataxia were noted in females beginning on day 2 and continuing through day 9. Skin effects (i.e. erythema, oedema, desiccation, scabs, sores, sloughing and/or cracking) were present in both sexes beginning on day 1 and continuing through the 14-day observation period. Body weight gain over the observation period in both sexes was decreased when compared with historical control data. Necropsy of the decedent revealed stomach and urinary bladder changes (yellow fluid in the stomach, red-tinged fluid and red areas on the inner lining of the urinary bladder). Necropsy of the survivors revealed no gross changes.

The acute dermal median lethal dose (LD_{50}) for *p*,*p*′-dicofol was greater than 5000 mg/kg bw in male and female rats (Ferguson, Craig & Eberly, 2000a).

(c) Exposure by inhalation

In a study of acute toxicity after inhalation, two groups of 10 male and 10 female Crl:CD BR rats each received a single 4-hour nose-only exposure to liquid aerosols of dicofol (Kelthane technical miticide; purity 94.4%, < 0.1% DDT-related impurities). The animals were observed for signs of intoxication during the exposure period and for 14 days following the exposure. Body weights were monitored during the 14-day post-exposure period. All animals that died were necropsied immediately, and all survivors were necropsied at the end of the 14-day observation period. The mean analytical active ingredient concentrations of dicofol were 2.2 ± 0.6 mg/l and 5.0 ± mg/l, which corresponded to total technical concentrations of 2.4 ± 0.0 and 5.3 ± 1.2 mg/l, respectively. The aerosol particle size parameters were a mass median aerodynamic diameter of 3.0 µm, a mean geometric standard deviation of 2.0 µm and a mean respirable fraction of 56%.

Exposure of male and female rats to dicofol at a mean analytical concentration of 2.2 mg/l produced treatment-related signs of sensory, nasal and upper airway irritation and treatment-related observations of reddened paws. Exposure of male and female rats to dicofol at a mean analytical concentration of 5.0 mg/l produced death (2/10 males); treatment-related signs of sensory, nasal and upper airway irritation; treatment-related observations of reddened paws; ataxia, scant feed consumption and scant faeces secondary to respiratory distress; statistically significant decreases in body weight gain; and treatment-related necropsy observations of air-filled and distended gastrointestinal tract, distended abdomen and red-stained muzzle. The air in the digestive tract was a result of gasping produced by nasal irritation. Mouth breathing by an obligate nose-breather, such as the rat, causes the swallowing of air.

The estimated median lethal concentration (LC_{50}) for male and female rats was greater than 5.0 mg of active ingredient of dicofol (Kelthane technical miticide) per litre of air, which corresponded to a total concentration of 5.3 mg of dicofol per litre of air (Fisher & Hagan, 1987).

(d) Dermal irritation

In this study of primary dermal irritation, six adult male New Zealand White rabbits were dermally exposed to 0.5 g of dicofol (Kelthane technical miticide; purity 95.2%). Skin irritation was evaluated according to Draize criteria at approximately 1, 24, 48 and 72 hours and at 7 days after patch removal.

No mortality or clinical signs of systemic toxicity were observed.

Very slight to well-defined erythema was observed at 1 hour in two rabbits. Well-defined to moderate to severe erythema was noted in all rabbits from 24 to 72 hours. Erythema was no longer evident by 7 days. Very slight to severe oedema was present in all rabbits beginning at 1 hour and continuing through 72 hours. Oedema was no longer evident on day 7. The primary irritation index was 4.8: slight to moderate dermal irritant (Ferguson, Craig & Eberly, 2000b).

(e) Ocular irritation

Eye irritation of dicofol (Kelthane technical miticide; purity 95.2%) was assessed in six adult New Zealand White rabbits. Eye irritation was evaluated according to Draize criteria at approximately 1, 24, 48 and 72 hours after dosing. After the 24-hour observation, each eye (treated and control) was irrigated with 0.9% saline solution for approximately 60 seconds.

No mortality or clinical signs of systemic toxicity were observed. No corneal or iridal effects were observed during the study. Conjunctival effects were observed in all rabbits at 1 and 24 hours and in five rabbits at 48 hours and were no longer evident by 72 hours. Dicofol is a slight to moderate eye irritant (Ferguson, Craig & Eberly, 2000c).

Table 16. Experimental design

Group	Induction treatment	Number of animals		Induction phase	Challenge phase	Rechallenge phase
		Males	Females			
1	Naive control	5	5	None	Dicofol at 11.7% w/v a.i. in acetone	—
2	Vehicle control	5	5	80% v/v aqueous ethanol	Acetone and dicofol at 11.7% w/v a.i. in acetone	—
3	Dicofol	10	10	Dicofol at 4.2% w/v a.i. in 80% v/v aqueous ethanol	Dicofol at 11.7% w/v a.i. in acetone	Dicofol at 11.7% w/v a.i. in acetone
4	Naive rechallenge control	4	6	None	—	Dicofol at 11.7% w/v a.i. in acetone

From Bonin & Hazelton (1987)
a.i., active ingredient; v/v, volume per volume; w/v, weight per volume

(f) Sensitization

The delayed contact hypersensitivity potential of dicofol (Kelthane technical miticide; purity 93.3%) was tested in young adult Hartley guinea-pigs using a modified Buehler procedure. The experimental design is shown in Table 16. The study was carried out according to OECD Test Guideline 406.

Dinitrochlorobenzene (DNCB) was used for validation of the modified Buehler test. In this validation, five guinea-pigs of each sex were treated. Concentrations of DNCB at 1600 parts per million (ppm) in 80% aqueous ethanol and 800 ppm in acetone were applied for induction and challenge, respectively. Erythema reactions were seen in 8 of 10 animals at 24 hours of observation and in 7 of 10 animals at 48 hours of observation (Bernacki, 1994).

One group of 20 guinea-pigs received 10 6-hour induction doses (3 doses per week, for 3.5 weeks) of 0.4 ml of 4.2% weight per volume (w/v) a.i. dicofol in 80% volume per volume (v/v) aqueous ethanol. An additional group of 10 guinea-pigs was also treated with 80% v/v aqueous ethanol in the same manner and served as a vehicle control group. These two groups of animals and a group of 10 naive control guinea-pigs (i.e. receiving no induction treatments) were challenged 2 weeks after the last induction dose with 0.4 ml of 11.7% w/v a.i. dicofol in acetone. Erythema reactions were scored at 24 and 48 hours after the challenge exposure. Seven days after the primary challenge, the 20 dicofol-induced guinea-pigs, as well as a second group of naive control guinea-pigs, received a rechallenge dose of 11.7% w/v a.i. dicofol in acetone and were scored similarly.

The primary challenge with dicofol elicited erythema in 2 of 10, 0 of 10 and 6 of 20 guinea-pigs in the naive control, vehicle control and 4.2% w/v a.i. dicofol groups, respectively. No erythema was observed in guinea-pigs induced with acetone. Following the second challenge with dicofol, 0 of 10 and 4 of 20 guinea-pigs in the naive control and dicofol-induced groups exhibited erythema, respectively.

The observation of erythema in 2 of 10 animals in the naive control group during the primary challenge was considered incidental and atypical of this group. The low incidence of erythema in the dicofol-induced group following the challenge and rechallenge phases, although not statistically different from the control groups, was considered indicative of a weak contact hypersensitivity response.

On the basis of this study, dicofol (Kelthane technical miticide) produced equivocal contact hypersensitivity in guinea-pigs under the conditions of the study. However, the incidence of delayed contact hypersensitivity was minimal (Bonin & Hazelton, 1987; reported in Annex 1, reference *67*).

Table 17. Effect of dicofol on male and female mice receiving technical dicofol in the diet daily for 13 weeks

Group	Dietary concentration (ppm)	Effect
2	10	No effect
3	125	Slightly decreased body weight (by 6% in males and by 6% in females, compared with the respective controls); increased liver weights (in females by 20% for absolute and by 25% for relative); decreased absolute kidney weight (by 10% in males); increased hepatic MFO activity
4	250	Decreased body weight (by 5% in males and by 7–9% in females, compared with the respective controls); increased SGPT (by 78% in females and by 68% in males); increased absolute (by 27% in females and by 15% in males) and relative (by 36% in females and by 21% in males) liver weights; decreased absolute kidney weight (in females by 10%); increased hepatic MFO activity; centrilobular hypertrophy
5	500	Decreased body weight; increased SGPT and triglycerides (males); enlarged livers with prominent architecture, pale in colour (females); granular, dilated and/or pale kidneys (females); increased liver weight (females); decreased kidney weight (females); increased hepatic MFO activity; increased hepatic microsomal protein concentration (males); centrilobular hypertrophy; necrosis and vacuolation of hypertrophied hepatocytes; diffuse hypertrophy of cells of adrenal cortex (males); dilatation and degeneration of cortical tubules of kidneys (females)
6	1000	Decreased body weight; decreased feed consumption (females); decreased haemoglobin, haematocrit and red blood cells and increased platelets (females); shifts in white blood cell differential, slight to moderate degree of spherocytosis and/or microcytosis (females); increased SGPT, triglycerides, total protein, globulin and creatinine (females); increased albumin, SGOT and calcium (males); decreased glucose (males); enlarged livers with prominent architecture, pale in colour (females); granular, dilated and/or pale kidneys (females); increased liver weight, decreased kidney weight, increased hepatic MFO activity, increased hepatic microsomal protein concentration; centrilobular hypertrophy; necrosis and vacuolation of hypertrophied hepatocytes; diffuse hypertrophy of cells of adrenal cortex; dilatation and degeneration of cortical tubules of kidneys; hyperplasia of covering epithelial cells of the papilla; hyperplasia and necrosis of collecting duct epithelium; vacuolation of tubular epithelium in outer medulla and hyperplasia of urinary bladder (females)

From Goldman & Harris (1986)
MFO, mixed-function oxidase; SGOT, serum glutamic oxalacetic transaminase (or aspartate aminotransferase); SGPT, serum glutamic pyruvic transaminase (or alanine aminotransferase)

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

Groups of 10 CD-1 (ICR) mice of each sex received technical dicofol (purity 95.6%; < 0.1% DDT-related impurities) in the diet daily for 13 weeks at 0, 10, 125, 250, 500 or 1000 ppm (equal to 0, 1.6, 18, 38, 84 and 180 mg/kg bw per day for males and 0, 2.1, 29, 56, 110 and 190 mg/kg bw per day for females). This study followed the principles of GLP, and OECD Test Guideline 408 was applied. The effects are shown in Table 17.

The NOAEL was 125 ppm (equal to 18 mg/kg bw per day), based on increased alanine aminotransferase (ALT) activity and other liver effects at 250 ppm (equal to 38 mg/kg bw per day) (Goldman & Harris, 1986; reported in Annex 1, reference *67*).

Rats

Groups of 10 Crl-CD (SD) rats of each sex received technical dicofol (purity 95.6%, < 0.1% DDT-related impurities) in the diet daily for 13 weeks at 0, 1, 10, 100, 500 or 1500 ppm (equal to 0,

0.07, 0.64, 6.5, 32 and 96 mg/kg bw per day for males and 0, 0.08, 0.78, 7.8, 36 and 110 mg/kg bw per day for females). All rats were observed daily for signs of ill-health or reaction to treatment. Body weight and feed consumption were monitored weekly beginning 1 week prior to treatment. Physical examinations were performed weekly on all animals. After 3 and 13 weeks of treatment, animals (10 of each sex per group) were bled for haematology and clinical chemistry analysis. Urinalysis and serum corticosterone determination were performed during the 11th week of treatment. Ophthalmoscopic examination was performed after 13 weeks of treatment. After 3 months, the animals were killed and necropsied, selected organ weights were recorded and tissues were collected for histopathological evaluation. At necropsy, liver samples were collected from five rats of each sex per group and analysed for mixed-function oxidase (MFO) activity. In addition, tissue samples of fat, gonads and livers were collected at necropsy for analysis of DDT, DDE and dicofol (Kelthane).

Some of the effects are summarized in Table 18. Five of 10 male and 8 of 10 female rats fed diets containing 1500 ppm dicofol died (1 female killed moribund) during the treatment period. Pre-death clinical signs included scant, soft or mucus-containing faeces, lethargy and ataxia. Clinical signs of scant droppings, soft faeces and/or faeces with mucus were seen in females at 500 ppm. Mean body weights were significantly decreased in rats fed diets containing dicofol at 500 ppm (by 14% in males and by 25% in females on average) or 1500 ppm (by 22% in males and by 17.5% in females on average) throughout the testing period. Feed consumption in these groups was also significantly decreased.

After 3 and 13 weeks of treatment, significant changes were observed in the haematological parameters monitored at 500 and 1500 ppm dicofol (see Table 18). Changes in a number of clinical chemistry parameters were also seen at 500 and 1500 ppm after 3 and 13 weeks of treatment. At 3 weeks, these changes included increased serum cholesterol levels in both sexes at 1500 ppm and in females at 500 ppm; increased serum ALT level (by 41% in males) and increased gamma-glutamyltranspeptidase (GGT) level in both sexes at 1500 ppm; decreased serum aspartate aminotransferase (AST) activity at 1500 ppm (by 18% in males and by 22% in females); decreased triglyceride levels (by 44% and 65% in males at 500 and 1500 ppm, respectively) and glucose levels (both sexes) at 500 and 1500 ppm; increased creatinine level in females at 1500 ppm; and decreased serum calcium and inorganic phosphorus levels in males at 1500 ppm. After 13 weeks of treatment with dicofol, clinical chemistry changes included decreased triglyceride levels at 500 and 1500 ppm (by 45% and 47% in males, respectively); increased cholesterol levels at 500 and 1500 ppm (both sexes); increased levels of ALT (by 28% in males), alkaline phosphatase (females) and GGT (both sexes) at 1500 ppm; and decreased glucose levels at 500 ppm (females) and at 1500 ppm (both sexes). No adverse clinical chemistry effects were seen in either sex, at either time period, at doses up to and including 100 ppm.

There was no indication of treatment-related ocular effects. At dietary concentrations of 500 ppm (females) and 1500 ppm (both sexes), there was a significantly decreased serum corticosterone level.

Hepatic MFO activity was significantly increased in rats by dietary administration of dicofol. The increase was evident in both sexes at dietary concentrations of 100, 500 and 1500 ppm.

Gross observations made at necropsy included enlarged livers in both sexes at 1500 ppm, darkened livers in males at 500 ppm and in both sexes at 1500 ppm, and small spleens in females at 1500 ppm. Histomorphological changes of the liver, including centrilobular hepatocellular hypertrophy, minimal to slight in males and slight to moderate in females, were observed at 100 ppm and above.

Organ weight changes were seen following 3 months of treatment with dicofol. Liver (absolute and relative) weights were significantly increased in both sexes at the two highest concentrations as follows: in males, liver weight was increased by 10% (absolute) and 32% (relative) at 500 ppm and by 63% (absolute) and 134% (relative) at 1500 ppm. In females, liver weight was increased by 32% (absolute) and 53% (relative) at 500 ppm and by 122% (absolute) and 180% (relative) at 1500 ppm.

Table 18. Summary of the effects of dicofol given for 13 weeks in the diet of male and female rats

	0 ppm (vehicle)		1 ppm		10 ppm		100 ppm		500 ppm		1500 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
No. of deaths	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	5/10	8/10
Mean body weight (% change)	NS	NS	NS	NS	NS	NS	NS	NS	−14*	−25*	−22*	−17*
Feed consumption (% change)	NS	NS	NS	NS	NS	NS	NS	NS	−20*	−14*	−24*	−25*
Clinical signs: scant droppings, soft faeces, faeces with mucus	No	No	No	No	No	No	No	No	Yes	Yes	Yes	Yes
Haematology and clinical chemistry at 3 weeks (% change)												
- MCV	NS	NS	NS	NS	NS	NS	NS	NS	−2**	NS	−2**	−4**
- MCH	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	−6**	NS
- HCT	NS	NS	NS	NS	NS	NS	NS	NS	NS	+7**	NS	NS
- PLAT	NS	NS	NS	NS	NS	NS	NS	NS	NS	+24**	+53**	+54**
- AST	NS	NS	NS	NS	NS	NS	NS	NS	NS	−25*	−18*	NS
- ALT	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	+41*	NS
- TRIG	NS	NS	NS	NS	NS	NS	NS	NS	−44*	+94*	−65*	NS
- CHOL	NS	NS	NS	NS	NS	NS	NS	NS	NS	+45*	+96*	+164*
Haematology and clinical chemistry at 13 weeks (% change)												
- MCV	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	−3**	−10**
- MCH	NS	NS	NS	NS	NS	NS	NS	NS	NS	−5**	−6**	−10**
- HCT	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	−5**	− 9**
- PLAT	NS	NS	NS	NS	NS	NS	NS	NS	NS	+23**	+53**	+68**
- AST	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
- ALT	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	+28*	NS

Table 18 (continued)

	0 ppm (vehicle)		1 ppm		10 ppm		100 ppm		500 ppm		1500 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
- TRIG	NS	NS	NS	NS	NS	NS	NS	NS	−45*	NS	−47*	NS
- CHOL	NS	NS	NS	NS	NS	NS	NS	NS	+47*	+34*	+134*	+196*
Hepatic MFO activity												
- AP (per total liver)	NS	NS	NS	NS	NS	NS	+1.7-fold*	+2.0-fold*	+2.25-fold*	+3.0-fold*	+2.2-fold*	+4.9-fold*
- AH (per total liver)	NS	NS	NS	NS	NS	NS	NS	+1.5-fold*	+1.79-fold*	+1.59-fold*	NS	+1.7-fold*
- AH (per gram liver)	NS	NS	NS	NS	NS	NS	NS	+1.4-fold*	+1.7-fold*	+1.2-fold	NS	NS
Microsomal protein (mg/g liver)	NS	NS	NS	NS	NS	NS	NS	NS	+1.4-fold*	NS	+1.4-fold*	NS
Organ weights (% change)												
- absolute liver weight	NS	NS	NS	NS	NS	NS	NS	NS	+10*	+32*	+63*	+122*
- relative liver weight	NS	NS	NS	NS	NS	NS	NS	+10*	+32*	+53*	+134*	+180*
- absolute kidney weight	NS	NS	NS	NS	NS	NS	NS	NS	−11*	+21*	NS	NS
- relative kidney weight	NS	NS	NS	NS	NS	NS	NS	+9*	NS	+40*	+44*	+34*
- absolute adrenal weight	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	+52*	NS
- relative adrenal weight	NS	NS	NS	NS	NS	NS	NS	NS	NS	+28*	+117*	NS
Histopathology changes												
- thyroid follicular hypertrophy (minimal to moderate)	0/10	0/10	0/10	0/10	4/10	0/10	6/10	0/10	7/10	5/10	8/10	6/10

From Goldman, Bernacki & Quinn (1986)

AH, aniline-4-hydroxylase; ALT, alanine aminotransferase; AP, aminopyrine *N*-demethylase; AST, aspartate aminotransferase, CHOL, cholesterol; HCT, haematocrit; MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume; MFO, mixed-function oxidase; PLAT, platelets; TRIG, triglycerides; NS, not significant; * $P < 0.05$; ** $P < 0.01$

Relative liver weight was increased by 10% at 100 ppm in females. Relative kidney weights were increased at 500 ppm (by 40% in females) and 1500 ppm (by 44% and 34% in males and females, respectively). Adrenal weights (absolute and relative) were increased in males at 1500 ppm and in females (relative) at 500 ppm.

The incidence and severity of thyroid follicular cell hypertrophy (minimal to moderate) were increased in males at 10 ppm and above and in females at 500 and 1500 ppm. The pathologist considered the thyroid finding of uncertain significance because it is a relatively nonspecific change that might be associated with the effect on the liver.

On the basis of this study, the NOAEL was 100 ppm (equal to 6.5 mg/kg bw per day), based on the reduction of mean body weight at 500 ppm (equal to 32 mg/kg bw per day) in both sexes **(**Goldman, Bernacki & Quinn 1986; reported in Annex 1, reference *67*).

Dogs

Groups of six Beagle dogs of each sex received technical dicofol (purity 93.3%; < 0.1% DDT-related impurities) in the diet daily for 13 weeks at 0, 10, 100, 300 or 1000 ppm (equal to 0, 0.29, 3.3, 9.9 and 26 mg/kg bw per day for males and 0, 0.31, 3.4, 9.8 and 27 mg/kg bw per day for females). All animals were observed twice daily for clinical signs of toxicity and mortality. Detailed examinations were conducted weekly during the time of individual body weight measurements. Individual body weights were determined during the pre-test period, on "0" (day of initiation), weekly during the 91-day experimental period and prior to termination. Feed consumption was measured daily during the 13 weeks. Blood biochemical and haematological parameters were determined twice on each dog prior to the start of the study, at week 4 and prior to termination. Urinalysis parameters were determined twice on each dog during the pre-test period and on all controls and surviving dogs at the 300 and 1000 ppm dose levels prior to the termination of the study.

Special biochemical assays, consisting of determination of blood cortisol levels after adrenocorticotropic hormone (ACTH) injections, were conducted on each dog prior to the study, at 4 weeks and prior to termination. Physiological measurements consisting of electrocardiograph (ECG) determinations and heart rates were conducted on each dog prior to the study, at 4 weeks and prior to termination. Rectal temperature was determined pre-test, at weeks 5 and 8 and prior to termination. Ophthalmological examinations were conducted on each animal during the pre-test period and prior to termination. Animals were necropsied at the end of the 91-day experimental period; brain, adrenals, gonads, heart, kidneys, liver, pituitary, thyroid/parathyroid and spleen were weighed for the calculation of mean absolute organ weights, as well as mean organ to body weight and organ to brain weight ratios. Tissues from each animal were taken and preserved for pathology assessment.

The high concentration of 1000 ppm caused death in five of six male and five of six female animals. Clinical signs considered related to treatment, including laboured breathing, inactivity, dehydration, red-tinged diarrhoea, incoordination and excessive salivation, were observed at 300 and 1000 ppm. Feed consumption decreased markedly 1–4 days prior to death in 1000 ppm animals found dead or sacrificed as moribund. Clinical parameters were determined in two 1000 ppm males and two 1000 ppm females on the day they were sacrificed in moribund condition. At the highest dose tested, ALT activity was increased about 3-fold in females and 6-fold in males, and serum alkaline phosphatase activity was increased about 10-fold and 8-fold in females and males, respectively. At 300 ppm and below, body weight and feed consumption were unaffected in both male and female dogs. In males, protein, albumin, calcium and cholesterol levels were decreased and serum alkaline phosphatase activity was slightly, but not significantly, elevated. Serum alkaline phosphatase activity was significantly elevated in females at the termination of the study, and mean calcium and albumin levels were significantly decreased in females. No effects were seen at 10 or 100 ppm.

Evaluation of the ECG revealed consistent changes characterized as prolongation of the QT and PR intervals, which were seen predominately in the 300 and 1000 ppm groups

Dicofol depressed the adrenal response to ACTH injection. Baseline cortisol blood levels were normal, but cortisol response to ACTH challenge (20 units of ACTH; cortisol measured 30 and 90 minutes after challenge) was markedly decreased (50–75%) in both sexes at 100 ppm and above. The adrenals, however, appeared normal when examined grossly and microscopically.

A decrease in spermatogenesis was observed in five 1000 ppm, three 300 ppm, one 100 ppm and one 10 ppm male rat. The latter two cases were considered to be unrelated to treatment.

Determination of organ weights and calculation of organ to body weight and organ to brain weight ratios revealed statistically significant increased mean hepatic weights of males at 300 ppm (by 37%) and 1000 ppm (by 22%) and increased hepatic weights of the surviving 1000 ppm females (by 94%).

Gross lesions, characterized as varying degrees of haemorrhage, congestion and luminal blood in the gastrointestinal tract, as well as icterus of the abdominal skin and of the mucous membrane of the sclera, oral cavity and vagina (females only), were detected in 1000 ppm males and females.

Microscopic examination of tissues revealed lesions in the liver, testes (males only) and heart in high-dose (1000 ppm) males and females. Gastrointestinal haemorrhagic enteritis and congestion were observed only in 1000 ppm males and females found dead or sacrificed as moribund, but were not seen in survivors at this level or in the animals at 300 ppm.

On the basis of this study, the NOAEL was 10 ppm (equal to 0.29 mg/kg bw per day), based on reduced cortisol response to ACTH challenge at 100 ppm (equal to 3.3 mg/kg bw per day) (Shellenberger, 1986; reported in Annex 1, reference *67*).

Groups of six Beagle dogs of each sex received technical dicofol (purity 93.3%; < 0.1% DDT-related impurities) in the diet daily for 52 weeks at 0, 5, 30 or 180 ppm (equal to 0, 0.12, 0.82 and 5.7 mg/kg bw per day for males and 0, 0.13, 0.85 and 5.4 mg/kg bw per day for females). All animals were observed twice daily for clinical signs of toxicity and mortality. Detailed examinations were conducted weekly. Individual body weights were determined during the pre-test period, on day "0" (day of initiation) and weekly thereafter. Feed consumption was determined daily. Blood haematological and biochemical parameters were determined twice on each dog prior to the start of the study, at weeks 13, 26 and 39 and prior to termination. Urinalysis parameters were determined on each dog during the pre-test period, at week 26 and prior to termination. Special biochemical assays, consisting of determination of blood cortisol levels after ACTH injections, were conducted on each dog prior to the study and during weeks 12, 25 and 51. Physiological measurements consisting of ECG determinations and heart rates were conducted on each dog prior to the study and on all dogs except the high-dose recovery group during weeks 1, 4, 13 and 26 and prior to termination. Rectal temperature was determined pre-test, at weeks 14 and 26 and prior to termination.

Adverse findings occurred only at the high dose and were more prominent in male than in female dogs. In male dogs receiving 180 ppm dicofol, serum alkaline phosphatase activity was increased by 228% at termination, and cholesterol levels were significantly increased (with a peak of 36% measured in week 26). Albumin level was significantly decreased in both male and female dogs (by about 13% and 10%, respectively). In female dogs in week 39, lactate dehydrogenase (LDH) activity was significantly increased by 115%. These changes were suggestive of mild liver injury in both sexes at the high dose. At the same time, mean organ weights remained unchanged. Liver to body weight ratios and liver to brain weight ratios were increased by 22% and 29% in males, respectively, and remained unchanged in females. Baseline cortisol blood levels were normal, but cortisol response to ACTH challenge (20 units of ACTH; cortisol measured 30 and 90 minutes after challenge) was markedly decreased (by about 50%) in high-dose males and females.

Minimal to mild hepatocellular hypertrophy was observed in five of six males and five of six females receiving the high dose compared with none in the control and low- and mid-dose groups. No treatment-related microscopic changes in the adrenal gland were found.

On the basis of this study, the NOAEL was 30 ppm (equal to 0.82 mg/kg bw per day), based on liver histological and clinical chemistry changes at 180 ppm (equal to 5.4 mg/kg bw per day) (Tegeris & Shellenberger, 1988; reported in Annex 1, reference *67*).

The overall NOAEL for the two oral dog studies was considered to be 30 ppm (equal to 0.82 mg/kg bw per day), with an overall lowest-observed-adverse-effect level (LOAEL) of 100 ppm (equal to 3.3 mg/kg bw per day).

(b) Dermal application

Dogs

Dicofol (Kelthane technical miticide) was formulated as Kelthane50 wettable powder (49.8% a.i.) and applied topically to male Beagle dogs for 13 weeks. Kelthane50 was used as the test article because the technical grade is a glassy solid, which precludes it from being dosed effectively by the dermal route, and the dermal absorption of the wettable powder formulation is expected to be representative of human exposure scenarios. Kelthane50 was moistened with tap water (contact volume of 1 ml/kg bw) and applied topically using a gauze patch (sized to over approximately 10% of the total body surface area) to the shaved intact skin of five groups of six male dogs per group at 0, 3, 10, 20 or 75 mg/kg bw per day (expressed as active ingredient). The application sites were occluded for 6 hours/day, 5 days/week, over the 13-week period, testing a total of 62 applications. After the 6-hour exposure, the occluded dressing was removed, and the exposure site of each animal was washed with a 1% Ivory Soap solution. The exposure sites were wiped with paper towels saturated with tap water and gently blotted dry with paper towels. The 0 mg/kg bw (control) group was treated in a manner that was identical to the treatment of animals in all other groups, except that they were dosed with tap water only (i.e. 1 ml/kg bw). This study was performed according to OECD Test Guideline 411 and complied with GLP.

All dogs were observed daily for signs of ill-health or reaction to treatment. Feed consumption was determined daily for all animals beginning 2 weeks prior to treatment and throughout the treatment period. Physical examinations were performed, and body weights were determined weekly. Serum cortisol levels were measured on all dogs twice during the pre-test period (once each week) and after 1, 2, 4, 9, 11 and 13 weeks of treatment. At each bleeding interval, three samples were collected. Samples were collected 30 minutes before the dogs received an intramuscular injection of ACTH and at 30 and 90 minutes after they received the ACTH injection.

There were no mortalities or clinical signs indicative of systemic toxicity throughout the treatment period at doses up to and including 75 mg/kg bw per day. No treatment-related effects on body weight or feed consumption were seen at any dose.

There were no treatment-related effects on serum cortisol levels at any dose level or time period at the pre-ACTH bleeding interval (baseline serum cortisol). There were no treatment-related effects on serum cortisol levels at the 30- or 90-minute post-ACTH bleeding interval at any dose level up to and including 75 mg/kg bw per day during the first 2 weeks of treatment or at 3 mg/kg bw per day at any time during the study.

At 10 and 20 mg/kg bw per day, treatment-related decreases and/or trends were seen in serum cortisol levels and/or the change in serum cortisol levels at week 13 for the 30-minute post-ACTH interval and in serum cortisol levels and the change in serum cortisol levels at weeks 4, 9, 11 and/or 13 for the 90-minute post-ACTH interval. At 75 mg/kg bw per day, treatment-related decreases and/or trends were seen in serum cortisol levels and the change serum cortisol levels at week 9, 11 and 13 for the 30-minute post-ACTH interval and at weeks 4–13 for the 90-minute post-ACTH interval. The results are shown in Tables 19 and 20.

Table 19. Serum cortisol levels 30 minutes post-ACTH injection in male dogs dermally exposed to dicofol (Kelthane50) for 13 weeks

		Serum cortisol level (µg/gl)				
		Dose (mg a.i./kg bw per day)				
		0 (control)	3	10	20	75
Number of dogs	*N*	6	6	6	6	6
Pre-test, week 2	Mean ± SD	9.89 ± 0.91	10.52 ± 1.13	9.11 ± 1.23	10.53 ± 1.01	10.97 ± 1.82
Week 1	Mean ± SD	8.92 ± 1.47	7.28 ± 0.71	7.67 ± 3.40	9.55 ± 1.35	8.41 ± 1.7
	Adj. mean[a]	9.14	7.05	8.46	9.31	7.86
	P-value[b]					
Week 2	Mean ± SD	9.80 ± 1.92	9.23 ± 1.36	7.31 ± 1.71	9.80 ± 1.29	9.69 ± 1.67
	Adj. mean[a]	10.02	9.01	8.07	9.59	9.15
	P-value[b]					
Week 4	Mean ± SD	9.14 ± 1.10	9.12 ± 0.61	8.91 ± 3.23	9.73 ± 1.63	8.86 ± 2.32
	Adj. mean[a]	9.46	8.80	10.01	9.40	8.07
	P-value[b]					
Week 9	Mean ± SD	9.08 ± 1.06	8.94 ± 1.14	7.81 ± 2.79	8.08 ± 0.77	6.68 ± 1.78
	Adj. mean[a]	9.28	8.76	7.85	7.85	6.14
	P-value[b]	*				
Week 11	Mean ± SD	9.49 ± 1.14	8.94 ± 1.14	8.34 ± 2.51	9.74 ± 1.14	7.36 ± 1.82
	Adj. mean[a]	9.86	8.76	8.95	9.55	6.93
	P-value[b]	*				**
Week 13	Mean ± SD	9.44 ± 1.25	9.29 ± 1.15	8.45 ± 1.17	7.96 ± 0.83	7.34 ± 1.66
	Adj. mean[a]	9.66	9.06	9.21	7.73	6.81
	P-value[b]	*			**	**

From Ferguson, Morisson & Kemmerer (1998)
SD, standard deviation; * statistically significant differences among all groups (i.e. $P < 0.5$); ** statistically significant differences compared with control by Dunnett's criterion
[a] Adj. mean = mean values adjusted to second pre-test measurements using analysis of covariance model treating time as a nominal variable.
[b] *P*-value symbols under column heading "0 (control)" are based on the overall *F*-test from the analysis of covariance model.

Table 20. Serum cortisol levels 90 minutes post-ACTH injection in male dogs dermally exposed to dicofol (Kelthane50) for 13 weeks

		Serum cortisol level (µg/dl)				
		Dose (mg a.i./kg bw per day)				
		0 (control)	3	10	30	75
Number of dogs	*N*	6	6	6	6	6
Pre-test, week 2	Mean ± SD	15.28 ± 2.78	15.05 ± 2.11	14.64 ± 1.26	16.01 ± 1.86	15.81 ± 3.04
Week 1	Mean ± SD	15.59 ± 2.43	14.92 ± 1.10	12.66 ± 1.90	14.63 ± 2.32	14.39 ± 2.69
	Adj. mean[a]	15.61	15.05	12.96	14.36	14.20
	P-value[b]					
Week 2	Mean ± SD	14.67 ± 2.20	14.00 ± 0.78	13.28 ± 3.64	15.37 ± 1.59	12.79 ± 2.91
	Adj. mean[a]	14.71	14.14	13.62	15.06	12.58
	P-value[b]					

Table 20 (continued)

		Serum cortisol level (µg/dl)				
		Dose (mg a.i./kg bw per day)				
		0 (control)	3	10	30	75
Week 4	Mean ± SD	16.10 ± 2.80	14.64 ± 1.09	12.48 ± 2.61	13.13 ± 2.14	11.34 ± 2.34
	Adj. mean[a]	16.13	14.76	12.76	12.86	11.16
	P-value[b]	*		**		**
Week 9	Mean ± SD	16.07 ± 2.19	15.19 ± 0.63	13.01 ± 3.35	11.94 ± 1.01	11.09 ± 3.20
	Adj. mean[a]	16.11	15.37	13.43	11.56	10.82
	P-value[b]	*		**	**	**
Week 11	Mean ± SD	15.44 ± 1.46	14.40 ± 0.73	12.04 ± 2.62	13.19 ± 0.79	10.65 ± 2.34
	Adj. mean[a]	15.47	14.52	12.32	12.93	10.47
	P-value[b]	*		**	**	**
Week 13	Mean ± SD	16.17 ± 2.45	14.46 ± 1.54	12.98 ± 3.50	12.30 ± 1.46	9.82 ± 1.78
	Adj. mean[a]	16.21	14.63	13.36	11.95	9.58
	P-value[b]	*		**	**	**

From Ferguson, Morisson & Kemmerer (1998)

SD, standard deviation; * statistically significant differences among all groups (i.e. $P < 0.5$); ** statistically significant differences compared with control by Dunnett's criterion

[a] Adj. mean = mean values adjusted to second pre-test measurements using analysis of covariance model treating time as a nominal variable.

[b] *P*-value symbols under column heading "0 (control)" are based on the overall *F*-test from the analysis of covariance model.

On the basis of this study, the NOAEL was 3 mg/kg bw per day (expressed as active ingredient), based on the inhibition of the normal physiological increase in serum cortisol levels following ACTH stimulation at 10 mg/kg bw per day (Ferguson, Morisson & Kemmerer, 1998).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a carcinogenicity study, groups of 50 B6C3F1 mice of each sex were administered technical dicofol (purity 90%, < 1% DDT-related impurities) at 150–300 ppm (low-dose males), 300–600 ppm (high-dose males), 55–150 ppm (low-dose females) and 110–300 ppm (high-dose females) for 78–79 weeks, followed by an untreated period of 14–15 weeks. The time-weighted average dietary concentrations were 264 or 528 ppm (equivalent to 40 or 80 mg/kg bw per day) for males and 122 or 243 ppm (equivalent to 18 or 36 mg/kg bw per day) for females. The control group consisted of 29 mice of each sex. A description of the experimental design is shown in Table 21. The purity of the test material was initially reported as 40–60%, but later analyses of the test material (and a lot sample) indicated 87–93% purity (A.M. Rothman, personal communication, 1981; cited in Annex 1, reference *67*).

A statistically significant increase in the incidence of liver tumours, mainly carcinomas, was reported in high-dose male mice. At the request of the USEPA, Dr Maronpot of the National Toxicology Program (NTP) re-evaluated the liver pathology slides (letter from R.R. Maronpot to J.A. Moore, 5 April 1985; cited in Annex 1, reference 67). The major difference was the reclassification of most carcinomas to adenomas, which reflected a change in the conventions of the pathology community for classifying mouse liver tumours. The majority of tumours reported in 1978 by NCI (1978) were carcinomas. The incidences of mouse liver tumours from the two evaluations are presented in Table 22.

Based on the Maronpot re-evaluation, the increase in the hepatocellular adenomas and combined adenomas plus carcinomas was statistically significant by pairwise comparisons at both doses.

Table 21. Experimental design of long-term study of carcinogenicity in mice

Dose group	Initial group size	Dietary concentration (ppm)	Observation period		Time-weighted average concentration (ppm)[a]
			Treated (weeks)	Untreated (weeks)	
Males					
Control	20	0	0	91	0
Low dose	50	150	4		264
		200	15 (5–19)		
		250	14 (20–34)		
		300	45 (35–79)		
		0		14 (80–94)	
High dose	50	300	4		528
		400	15 (5–20)		
		500	14 (21–34)		
		600	45 (35–79)		
		0		14 (80–94)	
Female					
Control	20	0	0	91	0
Low dose	50	55	9		122
		85	10 (10–19)		
		100	14 (20–34)		
		150	45 (35–79)		
		0		15 (80–95)	
High dose	50	110	9		243
		170	10 (10–19)		
		200	14 (21–34)		
		300	45 (35–79)		
		0		15 (80–95)	

From NCI (1978)

[a] Time-weighted average concentration = Σ (concentration × weeks received) / Σ (weeks receiving chemical).

Table 22. The incidence of liver tumours in dicofol-treated male mice

	Number of tumour-bearing animals/number of animals examined (%)		
	Control	264 ppm	528 ppm
1978 NCI			
Hepatocellular adenomas	0/18 (0)	1/50 (2)	1/47 (2)
Hepatocellular carcinomas	3/18 (17)**	22/50 (44)*	35/47 (74)**
Combined tumours	3/18 (17)**	23/50 (46)*	36/47 (77)**
1985 NTP (Maronpot)			
Hepatocellular adenomas	0/18 (0)**	13/48 (27)**	23/47 (49)**
Hepatocellular carcinomas	2/18 (11)	12/48 (25)	9/47 (19)
Combined tumours	2/18 (11)**	25/48 (52)**	32/47 (68)**

From Nave & Hurt (2000)

NCI, National Cancer Institute; NTP, National Toxicology Program; significance of trend denoted at control; significance of pairwise comparison with control denoted at dose level; * $P < 0.05$; ** $P < 0.01$

There was also a statistically significant positive trend for adenomas and combined adenomas plus carcinomas. The incidence of hepatocellular carcinomas was increased at both doses compared with controls, but there was no pairwise or trend significance.

It was noted that the background incidence of hepatocellular adenomas in male B6C3F1 mice historically is generally high (Haseman, Huff & Borman, 1984). However, in this particular study, there were no hepatocellular adenomas in control animals.

Table 23. Experimental design of long-term study of carcinogenicity in rats

Dose group	Initial group size	Dietary concentration (ppm)	Observation period		Time-weighted average concentration (ppm)[a]
			Treated (weeks)	Untreated (weeks)	
Males					
Control	20	0	0	110	0
Low dose	50	380	19		471
		500	59 (20–78)		
		0		34 (79–112)	
High dose	50	760	19		942
		1000	59 (20–78)		
		0		34 (79–112)	
Females					
Control	20	0	0	110	0
Low dose	50	380	78		380
		0		34 (79–112)	
High dose	50	760	78		760
		0		34 (79–112)	

From NCI (1978)

[a] Time-weighted average concentration = Σ (concentration × weeks received) / Σ (weeks receiving chemical).

The compound did not affect the survival rates of the treated animals relative to controls. It produced no effect on the body weight of the treated males. There was a decrease in the body weights of high-dose females. This can be associated with the increased hepatic MFO activity, observed as a high-dose effect.

A NOAEL in male mice was not observed. The LOAEL in male mice was 264 ppm (equivalent to 40 mg/kg bw per day), based on the increase in hepatocellular adenomas (NCI, 1978; reported in Annex 1, reference *67*; Nave & Hurt, 2000).

Rats

In a carcinogenicity study, groups of 50 Osborne-Mendel rats of each sex were administered technical dicofol (purity 90%, < 1% DDT-related impurities) in the diet daily for 78 weeks, followed by a basal diet during a 34-week observation period. A description of the experimental design is shown in Table 23. Male rats received time-weighted average dietary concentrations of 471 or 942 ppm, and female rats received constant dietary concentrations of 380 or 760 ppm (equivalent to 24 and 47 mg/kg bw per day for males and 19 and 38 mg/kg bw per day for females). Groups of 20 male and 20 female control rats received untreated diets for 110 weeks.

Survival rates at 100 weeks were 55%, 64% and 72% for males and 80%, 92% and 88% for females in the control, low-dose and high-dose groups, respectively. Body weights of low- and high-dose males and females were lower than control weights throughout the treatment period. Feed consumption data were not reported. No treatment-related clinical signs were observed. No neoplastic or non-neoplastic lesions were associated with dicofol treatment.

The NOAEL in this study was 760 ppm (equivalent to 38 mg/kg bw per day), the highest dose tested (NCI, 1978; reported in Annex 1, reference *67*).

In a 24-month toxicity and carcinogenicity study, dicofol (Kelthane technical miticide; 93.3% a.i. and < 0.1% DDT-related impurities) was administered in the diet to four groups (100 of each sex per group) of CRL:CD BR rats for up to 24 months at dietary concentrations of 0 (control), 5, 50 or

Table 24. Experimental design of 24-month toxicity and carcinogenicity study in rats

Group	Compound	Dietary concentration (ppm a.i.)	Total no. of rats[a]	Number of rats killed at month				
				3	5[b]	12	16	24[c]
1	Dicofol	0	200	20	20	20	20	120
2	Dicofol	5	200	20	20	20	20	120
3	Dicofol	50	200	20	20	20	20	120
4	Dicofol	250	200	20	20	20	20	120

From Hazelton & Harris (1989)

[a] Number of rats was equally divided between sexes.

[b] Rats were placed on diets at the indicated level for 3 months and then placed on control diet until killed.

[c] All survivors at 24 months were killed.

250 ppm a.i. (equal to 0, 0.22, 2.2 and 11 mg/kg bw per day for males and 0, 0.27, 2.7 and 14 mg/kg bw per day for females). A description of the experimental design is shown in Table 24. The study was carried out according to OECD Test Guideline 453 and GLP requirements.

All rats were observed daily for signs of ill-health or reaction to treatment. Physical examinations were performed and body weight and feed consumption were monitored weekly for the first 14 weeks of dosing and every other week thereafter. Feed efficiency was monitored during the first 13 weeks of the study (i.e. during the growing phase of the animals). Ophthalmoscopic examinations were performed on all surviving rats from groups 1 and 4 at 12 and 24 months. Serum corticosterone concentrations were monitored in 10 rats of each sex per group after 2, 5, 11, 17 and 23 months. Urinalysis parameters were monitored in 10 rats of each sex per group from groups 1 and 4 after 2, 5 and 11 months and in 10 rats of each sex from all groups after 17 and 23 months. After 3, 6, 12, 18 and 24 months, 10 rats of each sex per dose were bled for clinical chemistry and haematology analysis. Additional serum samples were collected at the 3-month period for analysis of T_3, T_4 and TSH concentrations to monitor thyroid function. Also at the 3-month interval, 10 animals of each sex per group were removed from treatment, placed on control diet and killed after 2 months of recovery. These animals were then necropsied, and selected organs were collected and saved for possible future histopathological evaluation. At 12- and 18-month intervals, those animals bled for haematology and clinical chemistry analysis were killed and necropsied, organ weights were recorded and tissues were collected for histopathological evaluation. In addition, liver samples from five rats of each sex per group were analysed for MFO activity at 3 and 12 months. All rats that died or were killed moribund were necropsied, and tissues were collected for histopathological examination. At 24 months, all surviving rats were killed and necropsied, organ weights were recorded (20 of each sex per group, when available) and tissues were collected for histopathological examination.

No treatment-related clinical signs were observed throughout the 24 months of dietary administration of dicofol. Survival of male and female rats in groups 2 (5 ppm) and 3 (50 ppm) and of male rats in group 4 (250 ppm) was comparable to that of controls. Group 4 (250 ppm) females exhibited increased survival when compared with control females. Decreases in body weight (15–28%) were observed in group 4 (250 ppm) males and females throughout the 24 months of treatment. Feed consumption was decreased in group 3 (50 ppm) females and group 4 (250 ppm) males and females. There were no treatment-related changes in serum T_3, T_4 and TSH concentrations, urinalysis parameters, serum corticosterone concentrations or haematology parameters when tested throughout the study. Serum triglyceride levels of group 4 (250 ppm) males and females were consistently lower at all time intervals when compared with the controls. This finding was considered secondary to the reduced body weight and feed consumption of this group. There were no other treatment-related changes in any clinical chemistry parameters.

Ophthalmological examinations at 12 and 24 months showed no indication of compound-related ocular disease in group 4 (250 ppm) rats when compared with the control group.

Increases in hepatic MFO activity were observed in groups 3 (50 ppm) and 4 (250 ppm) males and females after 3 and 12 months of treatment. Increases in relative liver weights (9–31%) were observed in groups 3 (50 ppm) and 4 (250 ppm) males and females. Gross pathology changes at necropsy were seen primarily in the liver (i.e. prominent lobular architecture) in group 4 (250 ppm). An irregular or thickened appearance of the urinary bladder was seen in group 4 (250 ppm) females at 24 months.

Treatment-related microscopic changes were observed in the liver in groups 3 (50 ppm) and 4 (250 ppm) males and females at 3, 12, 18 and 24 months, consisting predominantly of centrilobular hepatocellular hypertrophy, which was frequently accompanied by an increased amount of centrilobular or midzonal hepatocellular vacuolation. Increased incidences of multifocal or single-cell necrosis, focal eosinophilic cellular alterations or focal hepatocellular hyperplasia were also observed at these dose levels. Microscopic changes in the adrenal glands, consisting of diffuse vacuolation of the adrenal cortical cells, were observed in 50 ppm females at 18 months and in 250 ppm males and females at 3, 12, 18 and/or 24 months. Changes in the urinary bladder, consisting of a low, but increased, incidence of chronic cystitis, were seen in 250 ppm females at 24 months.

Dietary exposure to dicofol did not result in an increased incidence of neoplasms of any organ system in any of the compound-treated groups.

It was concluded that the NOAEL was 5 ppm (equal to 0.22 mg/kg bw per day in males and 0.27 mg/kg bw per day in females), based on histopathological changes in the liver and adrenal gland at 50 ppm (equal to 2.2 mg/kg bw per day in males and 2.7 mg/kg bw per day in females). There was no indication of an oncogenic effect at any dose level (Hazelton & Harris, 1989; Quinn & Hazelton, 1990; reported in Annex 1, reference *67*).

2.4 Genotoxicity[1]

The genotoxic potential of dicofol was investigated in a battery of in vitro studies of genotoxicity and one in vivo study of chromosomal aberrations in rat bone marrow (Table 25). Dicofol was negative in assays for point mutation, chromosomal aberration, unscheduled deoxyribonucleic acid (DNA) synthesis and sister chromatid exchange.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

Dicofol technical (purity 93.3%) was administered to Crl:CD BR rats over two generations (one- and two-litter study) at 0, 5, 25, 125 or 250 ppm in the diet (equal to 0, 0.5, 2.1, 10 and 21 mg/kg bw per day for males and 0, 0.5, 2.2, 11 and 18 mg/kg bw per day for females). The first parental (F_0 parental generation) animals were treated for 10 weeks prior to mating, during mating, during pregnancy and through weaning of the F_1 offspring. Selected F_1 offspring (F_1 parental generation) were treated during growth, mating, the production of two F_2 litters (F_{2a}, F_{2b}) and until the second F_2 litter was weaned. The effects of dicofol are summarized in Table 26.

During the pre-mating period and gestation, F_0 females receiving 125 or 250 ppm showed reduced body weight gain and feed consumption. Treatment-related histological changes were observed in the liver, ovaries and adrenal glands of F_0 and F_1 parental rats. The most prominent liver change was minimal to moderately severe hypertrophy of centrilobular hepatocytes accompanied by centrilobular to midzonal vacuolation in F_0 and F_1 parental males and females. The response was more severe in males than in females. The incidence in F_1 parental males was 0 out of 25, 1 out of

[1] Reported in Annex 1, reference 67.

Table 25. Studies on genotoxicity of dicofol

End-point	Test system	Concentration	Purity (%)	Results	Reference
In vitro					
Reverse mutation (Ames test)[a]	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537	5000, 2000, 500, 200, 50 µg/plate dissolved in DMSO	95.6	Negative[b]	Higginbotham & Byers (1985)
Reverse mutation (Ames test)[a]	*S. typhimurium* TA98, TA100, TA1535, TA1537	5000, 2000, 500, 200, 100, 50, 20 µg/plate dissolved in DMSO	95.6	Negative[c]	O'Neill (1993)
Reverse mutation (Ames test)[a]	*S. typhimurium* TA98, TA100, TA1535, TA1537	1000, 333, 100, 33, 10, 3.3 µg/plate dissolved in DMSO	Unspecified	Negative[c]	Mortelmans et al. (1986)
Reverse mutation (Ames test)[a]	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	1–1000 µg/plate dissolved in DMSO	89.9	Negative	Shirasu, Moriya & Ohta (1980)
Gene mutations (CHO/HGPRT mutation assay)[a]	Chinese hamster ovary cells (CHO-K_1-BH_4)	3, 4, 4.5, 5, 6 µg/ml –S9 activation in DMSO	95.6	Negative	Foxall (1986)
		10, 12, 17, 20 µg/ml +S9 activation in DMSO		Negative	
Escherichia coli mutation assay[a]	*E. coli*, WP2 hcr	1–5000 µg/plate dissolved in DMSO	89.9	Negative	Shirasu, Moriya & Ohta (1980)
Bacillus subtilis rec-assay	*B. subtilis*, H17, M45	20–2000 µg/disc dissolved in DMSO	89.9	Negative	Shirasu, Moriya & Ohta (1980)
Unscheduled DNA synthesis	Male CRCD rat primary culture hepatocytes	0.025–0.5 µg/ml in DMSO	95.6	Negative	Foxall & Byers (1986)
Sex-linked recessive lethal mutation	*Drosophila melanogaster*	10 000 ppm, feeding and injection	34.8	Negative	Woodruff et al. (1985)
Chromosomal aberration[a]	Chinese hamster ovary cells (CHO-W-Bl)	7.5–20 µg/ml −S9 activation in DMSO	95.6	Negative	Ivett & Myhr (1986)
		7.5–22.5 µg/ml +S9 activation in DMSO		Negative	
Sister chromatid exchange[a]	Chinese hamster ovary cells (CHO-W-Bl)	5–500 µg/ml	Unspecified	Negative	Galloway et al. (1987)
In vivo					
Chromosomal aberrations (mammalian bone marrow aberration test)	Male CRL:COBS-CD(SD) rat[d]	47.8–478 mg a.i./kg bw (single oral dose)	89.6	Negative[e]	Sames & Doolittle (1986)

DMSO, dimethyl sulfoxide; S9, 9000 × *g* supernatant from rat liver

[a] Both with and without metabolic activation.

[b] No positive control in non-activated assay.

[c] Positive control under non-activated conditions.

[d] Group of 30 male rats (10 per dose). Dicofol administered diluted in corn oil. Bone marrow collected at 6, 24 and 48 hours after administration. A dose-related incidence of clinical signs (passivity, diarrhoea, stained muzzle and ataxia) at 478.0 mg a.i./kg bw was observed in all three treated groups.

[e] No evidence presented (e.g. mitotic index) to demonstrate that test material reached the target tissue. A maximum tolerated dose may not have been used.

25, 14 out of 25, 24 out of 25 and 25 out of 25 in the 0, 5, 25, 125 and 250 ppm groups, respectively. Focal eosinophilic cellular alteration was increased in F_1 parental male (6/25) and female (8/25) rats at 250 ppm and in F_1 parental females at 125 ppm (6/25) compared with controls (1/25 in males; 0/25 in females). At 250 ppm, there was an increase in bile duct hyperplasia in F_0 and F_1 parental females. Vacuolation of the ovary was increased at 250 ppm in F_0 parental females and at 25 ppm and above

Table 26. Summary of the effects of dicofol in a two-generation reproduction study in rats

Dietary concentration (ppm)	Adults	Offspring
5	Males, F_0 and F_1 parental generation	Offspring
	- no effect	- F_{1a}, no effect
	Females, F_0 and F_1 parental generation	- F_{2a}, no effect
	- no effect	- F_{2b}, no effect
25	Males, F_0 and F_1 parental generation	Offspring
	- histopathological changes in liver	- F_{1a}, no effect
	Females, F_0 and F_1 parental generation	- F_{2a}, no effect
	- histopathological changes in liver	- F_{2b}, no effect
	Females, F_1 parental generation	
	- histopathological changes in ovaries	
125	Males, F_0 and F_1 parental generation	Offspring, F_{1a}
	- histopathological changes in liver	- no effect
	Females, F_0 parental generation	Offspring, F_{2a}
	- decreased body weight and feed consumption during the pre-breeding period and gestation	- increased number of litters with all offspring dying
	Females, F_0 and F_1 parental generation	- decreased viability index
	- histopathological changes in liver and adrenals	Offspring, F_{2b}
	Females, F_1 parental generation	- increased number of litters with all offspring dying
	- histopathological changes in ovaries	- decreased viability index
250	Males, F_0 parental generation	Offspring, F_{1a}
	- decreased body weight and feed consumption during the pre-breeding period	- decreased viability index
		- decreased offspring body weight
	Males, F_0 and F_1 parental generation	Offspring, F_{2a}
	- histopathological changes in liver	- increased number of litters with all offspring dying
	Females, F_0 parental generation	- increased number of litters with stillborn
	- decreased body weight during the pre-breeding period, gestation and lactation	- decreased viability index
	- decreased feed consumption during the pre-breeding period, gestation and lactation	- decreased offspring body weight
	Females, F_0 and F_1 parental generation	Offspring, F_{2b}
	- histopathological changes in liver, adrenals and ovaries	- increased number of litters with all offspring dying
		- decreased viability index

From Solomon & Kulwich (1991)

in F_1 parental females. The incidences in F_1 parental females were 1 out of 25, 1 out of 25, 6 out of 25, 5 out of 25 and 18 out of 25 in the 0, 5, 25, 125 and 250 ppm groups, respectively. The change was characterized by an increase in the size and/or number of vacuoles in the cytoplasm of ovarian stromal cells. The morphological change was described as compatible with enhanced steroidogenic activity. The incidence of hypertrophy and/or vacuolation of the adrenal cortex was increased in F_0 and F_1 parental females receiving 125 ppm (F_0, 7/25; F_1, 8/25) and 250 ppm (F_0, 23/25; F_1, 25/25) compared with controls (F_0 and F_1, 0/25). The change was characterized by diffuse enlargement and increased amounts of finely vacuolated cytoplasm or prominent large vacuoles in the cells of the inner cortex.

The reproductive performance of F_0 and F_1 parental rats was unaffected. Offspring toxicity was observed in F_1 and F_2 pups at 125 and 250 ppm. The viability index was slightly decreased (91%) (not statistically significant, but considered treatment related) in F_1 offspring at 125 ppm and statistically significant at 250 ppm. A treatment-related increase in the number of litters with no offspring surviving to day 21 postpartum at 125 and 250 ppm was noted. A treatment-related increase in the total number of stillborn and in the number of litters with stillborn at 250 ppm (control: 3 stillborn from three litters; 250 ppm: 26 stillborn from six litters) was also noted. The viability index in F_2 offspring was 76% at 125 ppm and 78% at 250 ppm. The effect was considered biologically significant. At 250 ppm, growth of F_1 and F_2 pups was reduced during lactation by 6% and 10%, respectively.

The NOAEL for reproductive toxicity was 25 ppm (equal to 2.1 mg/kg bw per day), based on decreased viability at 125 ppm (equal to 10 mg/kg bw per day). The NOAEL for parental toxicity was 5 ppm (equal to 0.5 mg/kg bw per day), based on histopathological changes in the liver and ovaries at 25 ppm (equal to 2.1 mg/kg bw per day). The NOAEL for offspring toxicity was 25 ppm (equal to 2.1 mg/kg bw per day), based on decreased viability index and increased number of litters with all offspring dying at 125 ppm (equal to 10 mg/kg bw per day) (Solomon & Kulwich, 1991; reported in Annex 1, reference *67*).

The effect of dietary administration of dicofol on reproduction and endocrine status was studied in one generation of Crl:CD BR VAF/Plus rats and their offspring. Thirty males and 40 females per group were exposed to dicofol at a concentration of 0, 5, 25 or 125 ppm (equal to 0, 0.3, 1.7 and 8.7 mg/kg bw per day in males and 0, 0.4, 2.0 and 9.8 mg/kg bw per day in females). Dicofol was administered to parental animals (F_0) beginning at approximately 6 weeks of age for 10 weeks prior to mating and throughout the mating, gestation and lactation periods. Exposure of offspring potentially occurred during gestation (transplacentally) and lactation (via the milk) and continued through the diet after weaning until sacrifice. Ten females from each group comprised a satellite group, used for analysis of dicofol and its metabolite FW-152 in serum, milk and neonatal tissues.

All animals were observed daily for viability and clinical signs of toxicity. Body weight and feed consumption were monitored in all animals throughout the study. Prior to mating, F_0 females were monitored for estrous cycling by daily examination of vaginal cytology for 21 days. Indices of fertility and reproductive success, including time to mating, mating and gestation indices, gestation length and lactation index, were evaluated. Viability, growth and clinical signs of offspring (F_1 animals) were monitored throughout the lactation period.

At weaning, one F_1 pup of each sex per litter was randomly selected for gross examination and histopathology. All tissues were examined in animals from the control and high-dose groups. At weaning, one F_1 pup of each sex per litter was selected for continued treatment and evaluation of sexual maturation (preputial separation in males; vaginal patency in females), sperm evaluation in males and estrous cycling in females.

F_0 males were necropsied upon completion of estrous cycle evaluation (approximately 70 days of age), and F_1 males at approximately 90–100 days of age. Sperm evaluation, including motility, morphology, and epididymal and testicular sperm counts, was performed for all F_0 and F_1 adult males at the time of necropsy. Selected tissues, including various reproductive tissues and known target organs, were weighed from all other animals and preserved for possible histopathology. Histopathological evaluation was performed for all tissues from all animals of the control and high-dose groups. Primary, growing and antral follicles were counted in ovaries from F_0 and F_1 adult females of the control and high-dose groups. In addition, reproductive tissues were examined from animals that did not mate, sire a litter, become pregnant or deliver healthy offspring.

Levels of dicofol and its metabolite FW-152 (*o,p′*- and *p,p′*- isomers of both) were determined in adult serum, milk, neonatal tissue and weanling serum samples. Serum was collected from F_0 females in the satellite groups during weeks 5 and 10 of treatment. Milk was collected on days 2 and

12 postpartum from all satellite group females that delivered litters. Pups were collected for analysis from each of these litters prior to nursing. Serum samples were collected from weanling F_1 animals selected for gross necropsy at day 21 postpartum.

Compound-related effects on organ weight and histopathological changes in the liver were observed only at 125 ppm. At 125 ppm, liver weight (absolute and relative to brain and/or body weight) was increased in males and females, paired kidney weights (absolute and relative to brain and body weights) were reduced in males only, and paired ovary weights (absolute and relative to body weight) were increased in females. A transient statistically significant decrease in body weight was observed in F_0 males at 125 ppm during weeks 3–7.

At 125 ppm, in F_1 animals at weaning, the predominant histopathological finding was vacuolation of centrilobular hepatocytes, which was observed in 27 of 29 males and 27 of 28 females and accompanied by hypertrophy of these same cells in 2 of 28 females. However, neither hyperplasia nor necroses were observed. In F_0 and F_1 adult animals of both sexes, hypertrophy with increased cytoplasmic eosinophils of a narrow band of hepatocytes surrounding the central vein was observed diffusely throughout all lobes of the liver in nearly all of the animals. There were no treatment-related histopathological findings in reproductive organs of any animals at any dietary concentration.

There were no treatment-related effects on mating performance (time to mating, mating index), fertility (fertility and gestation indices), gestation length or litter size. The sex ratio, growth and survival of offspring were unaffected by treatment. There were no treatment-related effects on estrous cyclicity of parental females or female offspring or on sperm counts, motility or morphology of parental males or male offspring. There were no effects on primordial, growing or antral follicle counts in either parental or offspring females. Sexual maturation of offspring was unaffected. There were no treatment-related gross or microscopic changes in reproductive or endocrine tissues of parental animals or weanling or adult offspring. An equivocal increase in ovary weights of weanling females was observed at 125 ppm, but it was not considered indicative of an endocrine-mediated effect due to the absence of histopathological changes or other associated effects.

p,*p*′-Dicofol was present in F_0 serum (0.07–1.2 ppm), milk (4.6–72 ppm), F_1 neonates (0.1–1.4 ppm) and weanling serum (0.24–1.25 ppm) samples of all dose groups. *p*,*p*′-FW-152 was quantifiable in F_0 serum at 125 ppm only (0.02 ppm) and neonates (0.05–0.28 ppm) and F_1 weanling serum (0.04–0.11 ppm) at 25 and 125 ppm. *p*,*p*′-Dicofol and *o*,*p*′-FW-152 levels were quantifiable only in milk samples. Increasing levels were observed with increasing dietary concentrations. Serum levels of analyses from F_0 females were similar at 5 and 10 weeks of treatment. The highest levels of all analytes were found in milk samples collected on day 2 postpartum, when all four analyses (*p*,*p*′-dicofol [4.6–72 ppm], *o*,*p*′-dicofol [0.03–0.10 ppm], *p*,*p*′-FW-152 [0.02–1.97 ppm] and *o*,*p*′-FW-152 [0.02–0.18 ppm]) were quantifiable in samples from all dose groups.

The NOAEL for parental toxicity was 25 ppm (equal to 1.7 and 2.0 mg/kg bw per day in F_0 males and unmated F_0 females, respectively), based on the transient decrease in body weight and organ weight changes in F_0 males and histopathological alterations in liver of both F_0 and F_1 adult males and females at 125 ppm (equal to 8.7 and 9.8 mg/kg bw per day in males and females, respectively) (Hoberman, 1997, 1998).

The NOAEL for reproductive and offspring toxicity was 125 ppm (equal to 8.7 mg/kg bw per day), the highest dose tested. This is confirmed by a supplementary study (Lomax, 1998), using the design of the original study (Hoberman, 1998), in which the numbers of primordial and growing follicles were counted in the ovaries of both F_0 (parental) and F_1 (offspring) adult animals in the control and high-dose (125 ppm) groups. This study was carried out in accordance with a request by the USEPA after its review of the original study, which contained follicle counts on primordial, growing and antral follicles enumerated individually. There were no treatment-related effects on primordial plus growing follicle counts in F_0 or F_1 animals exposed to 125 ppm dicofol in the diet. On the basis of these results, it was concluded that follicle count evaluation from F_0 and F_1 animals provided no

evidence of ovarian toxicity associated with exposure to 125 ppm dicofol under the conditions of this study (Lomax, 1998).

In a published study in male Wistar rats treated with dicofol (200, 300, 400 or 500 mg/kg bw per day) for 30 days, significant decreases in the weight of testes (by 23%), weight of the epididymis (by 28%) and the height of the Sertoli cells (by 47%) were observed in rats at the highest dose tested only. These were associated with some histopathological changes (Jadaramkunti & Kaliwal, 2002).

In another recently published study, the potential of dicofol and four other pesticides (dichlorvos, permethrin, endosulfan and dieldrin) to cause reproductive toxicity was investigated in rats dosed with these chemicals individually or in mixtures. Dicofol was dosed alone at 2.5 mg/kg (lowest-observed-effect level [LOEL]) and in a mixture at 0.22 mg/kg (NOAEL) in the diet for 8 weeks. A statistically significant reduction in the number of type A sperm (mobile with progressive trajectory) and the highest percentage of type C sperm (immobile) were measured in the animals exposed to dicofol alone, as well as exposed to the mixture of pesticides. However, no alterations in sperm morphology were observed in treated animals. Histopathological analyses of the testes and epididymis did not reveal any apparent alterations that could be attributed to pesticide exposure. Experimental groups did not differ significantly with respect to plasma level of testosterone, luteinizing hormone or follicle stimulating hormone. No changes in body weight or feed consumption were detected during the experiment. Additional studies need to be done to determine the molecular mechanisms underlying the actions of pesticides on reduced sperm motility (Perobelli at al., 2010).

(b) Developmental toxicity

Rats

The teratogenicity of dicofol was studied in Crl:COBS CD(SG)BR presumed pregnant rats. Dicofol was administered via gavage once daily, on days 6 through 15 of presumed gestation, to groups of 25 mated females at a dose of 0, 0.25, 2.5 or 25 mg/kg bw per day. Controls received corn oil. All dosages were given using a dose volume of 5 ml/kg bw. The rats were observed daily for clinical signs, abortion and/or death. Body weight and feed consumption were recorded on day 0 and days 6 through 20 of presumed gestation. On day 20, fetuses were delivered by caesarean section, and uterine examination was performed. Fetuses were examined for external, skeletal and visceral abnormalities.

No adult rats died during this study. Compared with control values, significant ($P < 0.5–0.01$) dose-dependent increases in the incidence of salivation occurred in rats dosed with dicofol at 2.5 (5/25 rats) and 25 (21/25 rats) mg/kg bw per day (Table 27). However, this effect might not be regarded as an effect of the compound itself, but more likely as an effect due to the mode of administration. Administration of dicofol at 25 mg/kg bw per day resulted in significant ($P < 0.01$) inhibition of average maternal body weight gain during the dosage interval, compared with the controls (Table 28). This correlates with a significant decrease in feed consumption and feed efficiency (g/kg bw per day) registered among the animals of this dose group (Table 29). Average liver to body weight ratios were significantly ($P < 0.01$) increased by about 7% in the high-dose group, compared with control values. Microscopic examination of the liver sections showed a minimal to slight hypertrophy of centrilobular hepatocytes.

Administration of the 0.25 and 2.5 mg/kg bw per day doses of dicofol did not affect average maternal body weight gain, liver weight, liver to body weight ratios or feed consumption/efficiency, compared with control values. At all dose levels, dicofol did not adversely affect the average number of live and dead fetuses or early and late resorptions. Dicofol did not affect fetal body weight or fetal

Table 27. Summary of daily incidence of adverse clinical signs, day 0 and days 6–15 of presumed gestation, female rats

	n	Salivation	Urine-stained abdominal fur	Chromo-dacryorrhoea	Red substance in cage pan	Alopecia	Lesion
0 mg/kg bw per day (vehicle)							
- no. of rats	25	0	0	0	1	4	0
- no. of days of observations	400	0	0	0	1	43	0
0.25 mg/kg bw per day							
- no. of rats	25	1	1	0	0	1	0
- no. of days of observations	400	1	1	0	0	11*	0
2.5 mg/kg bw per day							
- no. of rats	25	5*	0	1	0	1	1
- no. of days of observations	400	8**	0	14**	0	5**	4
25 mg/kg bw per day							
- no. of rats	25	21**	1	0	0	9	2
- no. of days of observations	400	57**	1	0	0	62*	24**

From Hoberman & Christian (1986a)
Statistically significant from vehicle control: * $P < 0.05$; ** $P < 0.01$

Table 28. Summary of maternal gestation body weight changes, day 0 and days 6–15 of presumed gestation[a]

		Maternal body weight changes (g)			
		Dose (mg/kg bw per day 6–15 of presumed gestation)			
		0 (vehicle)	0.25	2.5	25
No. of animals tested	*n*	25	25	25	25
No. of animals pregnant	*n* (%)	25 (100.0)	25 (100.0)	24 (96.4)	25 (100.0)
Days 0–6	X ± SD	+34.8 ± 8.6	+32.3 ± 9.2	+29.8 ± 9.2	+28.2 ± 11.1
Days 6–9	X ± SD	+9.5 ± 3.7	+9.8 ± 5.1	+10.2 ± 4.3	+2.0 ± 9.9**
Days 9–12	X ± SD	+16.1 ±5.4	+15.3 ± 4.2	+13.8 ± 3.7	+5.6 ± 7.2**
Days 12–16	X ± SD	+23.9 ± 7.4	+29.0 ± 7.7*	+25.1 ± 4.8	+27.3 ± 8.3
Days 16–20	X ± SD	+58.6 ± 14.9	+66.0 ± 10.0	+64.5 ± 10.4	+67.1 ± 12.4
Days 6–16	X ± SD	+49.5 ± 12.1	+54.1 ± 11.6	+49.2 ± 9.2	+34.9 ± 13.9**
Days 6–20	X ± SD	+108.1 ± 24.1	+114.8 ± 18.6	+113.7 ± 17.1	+100.0 ± 15.2
Days 0–20	X ± SD	+142.8 ± 29.7	+147.0 ± 21.9	+143.4 ± 18.7	+130.2 ± 20.2

From Hoberman & Christian (1986a)
SD, standard deviation; X, mean; statistically significant from vehicle control: * $P < 0.05$; ** $P < 0.01$
[a] This table is restricted to pregnant animals.

viability, compared with control values. No fetal alteration revealed by gross external, soft tissue or skeletal examination was attributed to the administration of dicofol to the dams.

On the basis of these data, the NOAEL for maternal toxicity was 2.5 mg/kg bw per day, based on the significant inhibition of maternal body weight gain during the dosage interval, decreased feed consumption and feed efficiency and the increase of liver to body weight ratios at 25 mg/kg bw per day. The increased incidence of salivation in 5 of 25 rats at a dose of 2.5 mg/kg bw per day and in 21 of 25 rats at 25 mg/kg bw per day would be considered more an effect due to the mode of

Table 29. Summary of maternal feed consumption, day 0 and days 6–15 of presumed gestation[a]

		Feed consumption (g/kg bw per day)			
		Dose group (mg/kg bw per day 6–15 of presumed gestation)			
		0 (vehicle)	0.25	2.5	25
No. of animals tested	*n*	25	25	25	25
No. of animals pregnant	*n* (%)	25 (100.0)	25 (100.0)	24 (96.4)	25 (100.0)
Days 0–6	X ± SD	95.5 ± 8.0	92.0 ± 13.2	92.2 ± 9.5	90.9 ± 10.9
Days 6–9	X ± SD	79.2 ± 7.1	77.8 ± 9.3	78.7 ± 6.2	65.8 ± 13.8**
Days 9–12	X ± SD	77.1 ± 7.6	77.2 ± 8.5	74.0 ± 5.8	53.4 ± 14.1**
Days 12–16	X ± SD	69.7 ± 8.2	74.5 ± 7.2*	68.8 ± 5.3	65.2 ± 9.5*
Days 16–20	X ± SD	75.5 ± 7.9	76.0 ± 7.2	76.4 ± 5.7	82.8 ± 7.1**
Days 6–16	X ± SD	74.5 ± 6.6	76.1 ± 9.3	73.1 ± 4.0	61.8 ± 8.9**
Days 6–20	X ± SD	74.6 ± 6.4	75.9 ± 7.5	74.0 ± 3.6	68.4 ± 6.1**
Days 0–20	X ± SD	77.0 ± 5.7	77.0 ± 8.0	75.8 ± 3.9	72.4 ± 5.8**

From Hoberman & Christian (1986a)

SD, standard deviation; X, mean; statistically significant from vehicle control: * $P < 0.05$; ** $P < 0.01$

[a] This table is restricted to pregnant animals. Data points for each animal are computed by dividing the main daily feed consumption for the interval by the mean body weight for the interval.

administration than an effect due to dicofol itself. Moreover, this is the only study in which salivation was reported.

The NOAEL for embryo toxicity and teratogenicity was 25 mg/kg bw per day, the highest dose tested (Hoberman & Christian, 1986a; reported in Annex 1, reference *67*).

Rabbits

Groups of 80 artificially inseminated pregnant New Zealand White rabbits (20 per group) received dicofol (purity 95.6%) in aqueous methylcellulose (1% v/v) at a dose of 0 (vehicle), 0.4, 4.0 or 40 mg/kg bw per day via oral gavage on days 7–19 of gestation. The rabbits were observed daily for clinical signs, mortality, abortion, premature delivery, body weight and feed consumption. On day 29 of gestation, fetuses were delivered by caesarean section, and uterus examination was performed. Fetuses were examined for external, skeletal and visceral abnormalities.

Administration of dicofol at a dose of 40 mg/kg bw per day resulted in abnormal faeces (dried, soft or liquid) compared with the controls (Table 30). This clinical observation presumably was inter-related with the decreased feed consumption, which was observed in the high-dose group (Table 31). Reduction and/or loss of weight gain for the high-dose group was statistically significant ($P < 0.05$–0.01), and, despite a significant ($P < 0.01$) rebound effect on body weight gain after completion of the dosage period, the overall body weight gain was depressed (42%) (Table 32).

The effect at 4.0 mg/kg bw per day was minimal and limited to a reversible inhibition and/or loss of body weight during the dosage period. The effect was not statistically significant ($P > 0.05$).

The highest dose of 40 mg/kg bw per day resulted in a biologically significant increase in the incidences of abortion (4/19, control 1/18) observed among rabbits with significant weight loss (Table 32).

The 40 mg/kg bw per day dicofol dose resulted in a significant ($P < 0.01$) 20% increase in the liver to body weight ratio, compared with the control values. Histopathological evaluation of liver tissue indicated that dose-dependent incidences of microscopic changes were observed in the livers of the 4.0 and 40 mg/kg bw per day dose group rabbits. These changes included accumulation of a densely eosinophilic, hyaline material in the cytoplasm of centrilobular hepatocytes. A very slightly higher incidence of a marked diffuse cytoplasmic vacuolation of hepatocytes occurred in the livers of

Table 30. Clinical signs and necropsy observations in rabbits administered dicofol

Dose (mg/kg bw per day 7–19 of presumed gestation)	*n*	No. pregnant (%)	No. died (%)	No. aborted (%)	No. delivered (%)	Abnormal faeces			Alopecia	Necropsy observation
						Total	Dried	Soft or liquid		Parovarian cyst(s)
0 (vehicle)										
- no. of rabbits	20	18 (90.0)	0	1 (5.6)	0	8	1	7	2	10
- no. of days	448	—	—	Day 17	—	15	1	14	13	—
0.4										
- no. of rabbits[a]	20	19 (95.0)	0	1 (5.6)	0	1*	0	1*	2	9
- no. of days	455	—	—	Day 24	—	3*	0	3*	6	—
4.0										
- no. of rabbits	20	18 (90.0)	1 (5.6)	0	0	5	1	4	9**	16[b]*
- no. of days	440	—	Day 9[b]	—	—	13	1	12	76**	—
40										
- no. of rabbits	20	19 (95.0)	0	4 (21)	2 (5.3)	16**	10**	12	6	13
- no. of days	444	—	—	Days 24, 24, 25, 27	Day 29	104**	36**	66**	61**	—

From Hoberman & Christian (1986b)

Statistically significant from vehicle control: * $P < 0.05$; ** $P < 0.01$

[a] One rabbit (100029) was removed and sacrificed on day 1 of presumed gestation due to an insemination accident. Another rabbit (100081) was added as a replacement on the 3rd day of insemination.

[b] The death of this rabbit was the result of a tracheal intubation perforation in the right diaphragmatic lobe of the lung.

40 mg/kg bw per day dose group rabbits, compared with control incidences. No microscopic changes that were judged to be dose related were observed in the livers of the 0.4 mg/kg bw per day dose group rabbits.

Administration of dicofol to pregnant rabbits did not affect fetal viability or average fetal body weight. All values for these parameters were similar and not significantly different ($P > 0.05$) among the four dose groups. No alterations revealed by external, soft tissue or skeletal examination of the fetuses were considered attributable to administration of these doses of dicofol to the does.

The NOAEL for maternal toxicity of dicofol was 4 mg/kg bw per day, based on abnormal faeces (dried, soft or liquid), decreased feed consumption, maternal weight loss, a significant increase in the incidences of abortion (4/19, control 1/18) and increased relative liver weights at 40 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 40 mg/kg bw per day, the highest dose tested (Hoberman & Christian, 1986b; reported in Annex 1, reference *67*).

2.6 Special studies

(a) Acute neurotoxicity

In a study of acute neurotoxicity, three groups of 10 male and 10 female Crl:CD BR VAF/Plus rats were given a single dose of dicofol (purity 95.5%) at 0, 3, 15, 75 or 350 mg/kg bw by gavage in corn oil (dose volume 5 ml/kg bw) and observed for the following 14 days. All rats were observed before the study start and daily throughout the study for any changes in clinical condition. Body weights and feed consumption were measured weekly throughout the study. All the rats were evaluated in functional observational battery (FOB) and motor activity tests (1.5-hour session on automated apparatus) during the week before the day of dosing and on days 1 (8 hours after dosing), 8 and 15. Six males and females per dose group were randomly selected, sacrificed and perfused

Table 31. Summary of maternal feed consumption in rabbits administered dicofol

		Maternal feed consumption (g/kg bw per day)			
		Dose[a] (mg/kg bw per day 7–19 of presumed gestation)			
		0	0.4	4.0	40
No. of animals tested	*n*	20	20	20	20
No. of animals pregnant	*n* (%)	18 (90.0)	19 (95.0)	18 (90.0)	19 (95.0)
Days 0–7	Mean ± SD	45.9 ± 4.2	45.8 ± 3.4	46.4 ± 3.1	46.9 ± 3.5
Days 7–10	Mean ± SD	43.6 ± 4.9	43.8 ± 3.0	42.7 ± 6.1 [17]	31.4 ± 11.6**
Days 10–13	Mean ± SD	41.8 ± 4.9	41.5 ± 7.0	43.0 ± 4.8 [17]	17.8 ± 13.0** [18]
Days 13–16	Mean ± SD	39.4 ± 5.7	37.9 ± 9.2 [18]	37.6 ± 6.7 [15]	11.7 ± 14.1**
Days 16–20	Mean ± SD	39.0 ± 9.0 [17]	38.7 ± 10.1	36.2 ± 6.5 [16]	9.9 ± 13.5**
Days 20–24	Mean ± SD	36.6 ± 5.9 [17]	37.2 ± 7.4 [17]	34.1 ± 8.6 [16]	25.2 ± 18.3 [17]
Days 20–29	Mean ± SD	29.9 ± 8.5 [17]	31.5 ± 8.6 [18]	28.9 ± 7.2 [16]	32.0 ± 12.3 [14]
Days 7–20	Mean ± SD	40.8 ± 5.1 [17]	40.4 ± 7.0	39.3 ± 3.8 [16]	17.4 ± 10.9**
Days 7–29	Mean ± SD	36.2 ± 4.5 [17]	37.5 ± 5.2 [18]	34.9 ± 3.8 [16]	25.6 ± 8.6** [14]
Days 0–29	Mean ± SD	38.2 ± 3.7 [17]	39.0 ± 4.2 [18]	37.3 ± 3.0 [16]	30.8 ± 6.1** [14]

From Hoberman & Christian (1986b)

SD, standard deviation; ** significantly different from vehicle control value at $P < 0.01$

[a] Test substance was administered on days 7 through 19 of presumed gestation. [ ], number of values averaged.

Table 32. Summary of maternal body weight changes in rabbits administered dicofol

		Maternal body weight changes (g)			
		Dose[a] (mg/kg bw per day 7–19 of presumed gestation)			
		0	0.4	4.0	40
No. of animals tested	*n*	20	20	20	20
No. of animals pregnant	*n* (%)	18 (90.0)	19 (95.0)	18 (90.0)	19 (95.0)
Days 0–7	Mean ± SD	+0.16 ± 0.07	+0.19 ± 0.07	+0.18 ± 0.08	+0.20 ± 0.06
Days 7–10	Mean ± SD	+0.02 ± 0.04	+0.02 ± 0.04	+0.00 ± 0.05 [17]	−0.11 ± 0.08**
Days 10–13	Mean ± SD	+0.03 ± 0.05	+0.06 ± 0.05	+0.07 ± 0.06 [17]	−0.05 ± 0.07** [18]
Days 13–16	Mean ± SD	+0.07 ± 0.05	+0.06 ± 0.04 [18]	+0.06 ± 0.05 [15]	−0.04 ± 0.11**
Days 16–20	Mean ± SD	+0.03 ± 0.05 [17]	+0.03 ± 0.05	−0.03 ± 0.09 [16]	−0.10 ± 0.13**
Days 20–24	Mean ± SD	+0.06 ± 0.06 [17]	+0.08 ± 0.06 [17]	+0.05 ± 0.06 [16]	+0.09 ± 0.18 [17]
Days 20–29	Mean ± SD	+0.06 ± 0.117 [17]	+0.08 ± 0.11 [18]	+0.07 ± 0.00 [16]	+0.22 ± 0.16** [14]
Days 7–20	Mean ± SD	+0.16 ± 0.09 [17]	+0.16 ± 0.00	+0.10 ± 0.09 [17]	−0.29 ± 0.25**
Days 7–29	Mean ± SD	+0.22 ± 0.22 [17]	+0.26 ± 0.13 [18]	+0.17 ± 0.12 [17]	+0.01 ± 0.25** [14]
Days 0–29	Mean ± SD	+0.38 ± 0.22 [17]	+0.46 ± 0.16 [18]	+0.35 ± 0.15 [17]	+0.22 ± 0.27* [14]

From Hoberman & Christian, 1986b

SD, standard deviation; significantly different from vehicle control value: * $P < 0.05$; ** $P < 0.01$

[a] This table is restricted to pregnant animals. Test substance was administered on days 7 through 19 of presumed gestation. Days refer to the days of presumed gestation. [ ], number of values averaged.

in situ for 14–19 days after dosing for possible neurohistological evaluation. Tissues from the rats selected in the control and the 350 mg/kg bw per day dose groups were further processed and evaluated microscopically.

No deaths occurred before scheduled sacrifices.

The 75 and 350 mg/kg bw per day doses of dicofol caused a significant reduction in body weight (5–10%) ($P < 0.05$ to $P < 0.01$) in male and female rats. Absolute (g/day) and relative (g/kg bw per day) feed consumption values were also significantly reduced ($P < 0.05$ to $P < 0.01$) in rats from both sexes in the 75 and 350 mg/kg bw per day dose groups. The changes observed after dicofol administration are presented in Tables 33 and 34.

On day 1 (8 hours after dosing) and the day after dosing, the number of rats with urine-stained and faeces-stained fur was significantly increased in the 350 mg/kg bw per day groups. On the day after treatment, the number of rears in the open field, performance in the air righting response and the total time spent in movement were significantly reduced in 350 mg/kg bw per day females.

On day 8 of the study, 3 of 10 high-dose female rats had urine-stained or faeces-stained fur, compared with 1 of 10 male rats from the same dose group. Four of 10 male and 4 of 10 female rats in this 350 mg/kg bw per day dose group were sleeping when observed in the cage. The total time spent in movement was also significantly reduced by 37% in the high-dose group female rats on day 8 of the study.

On day 15 of the study, landing foot splay (observed in the cage and in the open field) was significantly increased ($P < 0.05$) in female rats at 75 mg/kg bw per day and above. However, neurohistological evaluation of the peripheral nerves of rats in the high-dose group did not reveal evidence of pathology. The values from the motor activity test for number of movements and time spent in movement were similar among the four dose groups in both sexes on day 15 of the study.

A summary of the clinical observations of the male and female rats in the highest dose group, in which significant changes were observed, is shown in Table 35. The incidences of faeces-stained perianal fur or liquid faeces were significantly increased in the daily clinical observations of the 350 mg/kg bw per day dose group. These observations persisted in some rats until day 9 of the study. The incidences of tiptoe walk, ataxia and decreased motor activity were also significantly increased in the high-dose group. Chromodacryorrhoea occurred in single rats in each of the 75 and 350 mg/kg bw per day dose groups. Other clinical observations that occurred only in one or two rats of the high-dose group included impaired righting reflex, urine-stained abdominal fur, colourless faeces and localized alopecia.

No gross lesions were revealed by necropsy. The neurohistological evaluation of rats selected from the high-dose and control groups did not reveal pathology related to the test substance. Brain weights and the brain weight to terminal body weight ratios were similar among the four dose groups.

The NOAEL was 15 mg/kg bw per day, based on reduction of body weight and feed consumption at 75 mg/kg bw per day. Effects of the test substance were not evident 2 weeks after dosing. The neurohistological evaluation of the 350 mg/kg bw per day dose group rats did not reveal any pathology related to the test substance.

Dicofol does not appear to be a specific or selective neurotoxicant, because signs of behavioural dysfunction occurred at doses that also caused changes in body weight and feed consumption values and other signs of systemic toxicity (Foss, 1992).

(b) Short-term study of neurotoxicity

In a 90-day study of neurotoxicity performed in accordance with USEPA GLP standards, groups of 10 male and 10 female Crl:CD(SD)IGS BR rats were given diets containing dicofol (purity 95.5%) at a concentration of 0, 5, 100 or 500 ppm (equal to 0, 0.2, 6.7 and 33.3 mg/kg bw per day).

Table 33. Observed changes on day 1 (8 hours after dosing) and the day after dosing (day 2) in rats administered a single oral dose of dicofol

	0 mg/kg bw per day (vehicle)		15 mg/kg bw per day		75 mg/kg bw per day		350 mg/kg bw per day	
	Males	Females	Males	Females	Males	Females	Males	Females
Body weight	NS	NS	NS	NS	↓5%	↓5%	↓10%	↓10%
Absolute feed consumption	NS	NS	NS	NS	↓27%	↓44%	↓81%	↓76%
Relative feed consumption	NS	NS	NS	NS	↓26%	↓43%	↓80%	↓75%
Urine-stained and faeces-stained fur	0/10	0/10	1/10	0/10	3/10	0/10	8/10**	7/10**
Rears	NS	NS	NS	NS	NS	NS	NS	↓44%*
Air righting response								
- lands with all feet on the ground	7/10	9/10	9/10	7/10	7/10	7/10	2/10	3/10*
- uncoordinated landing	1/10	1/10	1/10	3/10	3/10	3/10	5/10	5/10*
- lands on back	0/10	0/10	0/10	0/10	0/10	0/10	3/10	2/10
Ataxia	0/10	0/10	0/10	0/10	0/10	0/10	3/10	2/10
Motor activity								
Day 1 (8 h after dosing)								
- number of movements	NS	NS	NS	NS	NS	NS	NS	NS
- time spent in movement	NS	NS	NS	NS	NS	NS	NS	↓38%*
Day 2								
- number of movements	NS	NS	NS	NS	NS	NS	NS	↓60%**
- time spent in movement	NS	NS	NS	NS	NS	NS	↓65%**	↓70%**

From Foss (1992)
NS, not significantly different; significantly different from the vehicle control group values: * $P < 0.05$; ** $P < 0.01$

The rats were observed for viability at least twice per day and for clinical signs of toxicity and general health weekly during the acclimation period and daily after the start of the pre-exposure behavioural testing. Body weight and feed consumption were recorded weekly and on the days of FOB and motor activity testing. Assessments of FOB and motor activity were conducted on all rats before exposure (baseline) and during weeks 4, 8 and 13. The rats were sacrificed and perfused in situ after 91 and 95 days of test diet exposure. Six rats of each sex per group were randomly chosen for neurohistological examination. Tissues from the rats selected in the control and high dietary concentration groups were further processed and evaluated microscopically.

No rats died before the scheduled sacrifices.

The changes observed in weeks 4, 8 and 13 are summarized in Table 36.

Body weight gains in the male rats were generally reduced by 38% ($P < 0.05$) on days 57–64 in the 100 ppm group and significantly reduced by 26% for females and by 27% for males ($P < 0.01$) in the 500 ppm group throughout the exposure period. The 500 ppm group female rats had significant

Table 34. Observed changes on day 8 in rats administered a single oral dose of dicofol

	0 mg/kg bw per day (vehicle)		15 mg/kg bw per day		75 mg/kg bw per day		350 mg/kg bw per day	
	Males	Females	Males	Females	Males	Females	Males	Females
Body weight	NS	NS	NS	NS	NS	NS	↓5%**	NS
Absolute feed consumption	NS	NS	NS	NS	↓16%**	NS	↓11%*	↑28%**
Relative feed consumption	NS	NS	NS	NS	↓13%**	NS	NS	↑32%**
Urine-stained and faeces-stained fur	0/10	0/10	0/10	0/10	0/10	0/10	1/10	3/10
Rears	NS	NS	NS	NS	NS	↓25%*	NS	NS
Air righting response								
- lands with all feet on the ground	5/10	9/10	9/10	9/10	10/10	7/10	8/10	7/10
- uncoordinated landing	5/10	1/10	1/10	1/10	0/10	3/10	2/10	3/10
- lands on back	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Ataxia	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Motor activity								
- number of movements	NS	NS	NS	NS	NS	NS	NS	NS
- time spent in movement	NS	NS	NS	NS	NS	NS	NS	↓37%*

From Foss (1992)
NS, not significantly different; significantly different from the vehicle control group values: * $P < 0.05$; ** $P < 0.01$

Table 35. Summary of clinical observations in male and female rats given a single oral dose of dicofol and observed for 14 days[a]

	0 mg/kg bw per day (vehicle)		350 mg/kg bw per day	
	Males	Females	Males	Females
Maximum possible incidence	178/10	178/10	181/10	181/10
Faeces-stained perianal fur	0/0	0/0	13/8**	33/10**
Soft or liquid faeces	0/0	0/0	9/6**	17/6**
Localized alopecia: limbs	0/0	0/0	17/2	7/1
Tiptoe walk	0/0	0/0	3/2	17/6**
Decreased motor activity	0/0	0/0	1/1	8/6**
Chromodacryorrhoea	2/2	0/0	0/0	5/4**

From Foss (1992)
** Significantly different ($P < 0.01$) from the vehicle control group value
[a] Statistical analysis of clinical observation data was restricted to the number of rats with observations. Maximum possible incidence: (day × rats)/number of rats examined per group.

body weight losses ($P < 0.05$ to $P < 0.01$) on days 1–8 and 71–78 of exposure and significantly reduced body weight gains (by 26%) ($P < 0.01$) for the exposure period (calculated as days 1–92 of exposure or as day 1 to termination). Body weights were generally significantly reduced ($P < 0.05$ to $P < 0.01$) in the 500 ppm group male and female rats on day 8 or 15 through day 92 of exposure.

Table 36. Observed changes in weeks 4, 8 and 13 in rats administered dicofol in the diet for 90 days

	% change relative to control							
	0 ppm (vehicle)		5 ppm		100 ppm		500 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
Body weight gain (days 1–92)	NS	NS	NS	NS	NS	NS	↓27**	↓26**
Terminal body weight							↓14**	↓10**
Week 4								
Body weight	NS	NS	NS	NS	NS	NS	↓10**	↓9*
Rears	NS	NS	NS	NS	NS	NS	NS	NS
Forelimb grip strength	NS	NS	NS	NS	NS	NS	↓16*	NS
Landing foot splay average	NS	NS	NS	NS	NS	NS	NS	NS
Motor activity								
- number of movements	NS	NS	NS	NS	NS	NS	↓38**	NS
- time spent in movement	NS	NS	NS	NS	NS	NS	↓48**	NS
Week 8								
Body weight	NS	NS	NS	NS	NS	NS	↓13**	↓12*
Rears	NS	NS	↓20*	↓30*	NS	NS	↓60**	↓36**
Forelimb grip strength	NS	NS	NS	NS	NS	NS	NS	NS
Landing foot splay average	NS	NS	NS	NS	NS	NS	NS	NS
Motor activity								
- number of movements	NS	NS	NS	NS	↓32*	↓40*	↓37*	NS
- time spent in movement	NS	NS	NS	NS	NS	↓44*	↓56**	NS
Week 13								
Body weight	NS	NS	NS	NS	NS	NS	↓13**	↓11*
Rears	NS	NS	NS	NS	↓61*	NS	↓67*	NS
Forelimb grip strength	NS	NS	NS	NS	NS	NS	NS	NS
Landing foot splay average	NS	NS	NS	NS	NS	NS	NS	↑29*
Motor activity								
- number of movements	NS	NS	NS	NS	↓27*	NS	↓37*	NS
- time spent in movement	NS	NS	NS	NS	↓35*	↓41*	↓43*	NS

From Foss (1993)

NS, not significantly different; significantly different from the vehicle control group values: * $P < 0.05$; ** $P < 0.01$

Absolute (g/day) feed consumption values in the 100 ppm group male rats were slightly reduced beginning on days 50–57 of exposure; this reduction was significant by 6% ($P < 0.05$) on days 85 and 92 of exposure. The 500 ppm group male and female rats had significantly reduced absolute feed consumption values, by 11% and 10%, respectively, throughout the exposure period.

The numbers of times rats were observed to rear in the open field during the examination were significantly reduced ($P < 0.05$ to $P < 0.01$) in the 500 ppm group male and female rats (by 60% and 36%, respectively) in week 8 of exposure and in the 100 and 500 ppm group male rats in week 13 of exposure (by 61% and 67%, respectively). Forelimb grip strength values were significantly reduced by 16% ($P < 0.05$) in the 500 ppm group male rats in week 4, but not in week 8 or week 13 of exposure, and landing foot splay was significantly increased by 29% ($P < 0.05$) in the 500 ppm group female rats in week 13 of exposure.

The total number of movements and the total time spent in movement were significantly reduced by 38% and 48% ($P < 0.01$), respectively, in the 500 ppm group male rats in week 4 of exposure. The 500 ppm group male rats also had differences in the patterns of group values across the measurement periods, with significant reductions ($P < 0.01$ to $P < 0.05$) in the number of movements and time spent in movement at specific intervals. When the male rats were tested in weeks 8 and 13 of exposure, the total number of movements and the total time spent in movement were reduced or significantly reduced ($P < 0.05$ to $P < 0.01$) in the 100 and 500 ppm groups. Motor activity parameters in the female rats were unaffected by exposure to dicofol.

No gross lesions revealed at necropsy were considered effects of the test substance. The neurohistological examination of rats selected from the control and 500 ppm groups did not reveal any pathology related to the test substance.

Terminal body weights were significantly reduced ($P < 0.01$) in the 500 ppm male and female rats to 86% and 90% of the control group values, respectively. In male rats, changes were observed mostly in the high-dose group (500 ppm). The ratios of liver weight to terminal body weight and to brain weight were increased in the 500 ppm male rats and in the 100 and 500 ppm female rats. Kidney weight and the ratios of kidney weight to terminal body weight were significantly increased in the 500 ppm group female rats.

The NOAEL was 5 ppm (equal to 0.2 mg/kg bw per day). The 100 and 500 ppm dietary concentrations affected parameters in the FOB, altered absolute and relative organ weights and reduced body weight, feed consumption values and motor activity. The neurohistological examination of rats at the highest dose tested, 500 ppm, did not reveal any pathology related to the test substance.

Dicofol does not appear to be a specific or selective neurotoxicant, because signs of behavioural dysfunction occurred at doses that also caused changes in organ weights, body weights and feed consumption values (Foss, 1993).

In a published study, the effect of dicofol (from Pestanal, Sigma-Aldrich, Steinheim, Germany) on in vivo release of dopamine and its metabolites dihydroxyphenylacetic acid and homovanillic acid in awake and freely moving female Sprague-Dawley rats ($n = 6$) was measured by brain microdialysis coupled to HPLC with electrochemical detection. Intrastriatal 60-minute infusion of dicofol at 1 mmol/l did not induce significant alterations in extracellular levels of dopamine and its metabolites. Dicofol does not exert a neurotoxic effect (Faro et al., 2009).

(c) Biochemical aspects of metabolites

The effect of technical dicofol (87.5% a.i.; 78.50% *p*,*p*′-dicofol, 13.11% *o*,*p*′-dicofol), *p*,*p*′-dicofol (99.7% a.i.), *o*,*p*′-dicofol (92% a.i.) and six structural analogues of *p*,*p*′-dicofol, which are each present as an impurity in technical dicofol (Kelthane technical), were investigated for their effect on liver MFO activity in male B6C3F1 mice. Groups of four mice were administered the test material in the diet daily for 2 weeks. The experimental design is shown in Table 37. Apart from MFO activity,

Table 37. Experimental design of study on dicofol and its structural analogues

Group	Test substance	Concentration of test substance in diet (ppm)[a]	Group	Test substance	Concentration of test substance in diet (ppm)[a]
1A	Vehicle	0	3A	Vehicle	0
1B	Dicofol technical	8	3B	*o,p′*-Dicofol	37.5
1C	Dicofol technical	25	3C	*p,p′*-ER-8	37.5
1D	Dicofol technical	80	3D	*o,p′*-ER-8	37.5
1E	Dicofol technical	250	3E	*o,p′*-DDE	37.5
1F	Dicofol technical	800	3F	*o,p′*-DDE	37.5
2A	Vehicle	0	4A	Vehicle	0
2B	*p,p′*-Dicofol	6	4B	*o,p′*-Dicofol	195
2C	*p,p′*-Dicofol	20	4C	*p,p′*-DCBP	20
2D	*p,p′*-Dicofol	63	4D	*p,p′*-DCBP	63
2E	*p,p′*-Dicofol	195	4E	*p,p′*-DCBP	175
2F	*p,p′*-Dicofol	625	4F	*o,p′*-DCBP	195

From Steigerwalt, Deckert & Longacre (1984a)

DCBP, dichlorobenzophenone; DDE, dichlorodiphenyldichloroethene; *o,p′*-ER-8, 1-chloro-1-(2-chlorophenyl)-1-(4-chlorophenyl)-2,2,2-trichloroethane; *p,p′*-ER-8, 1-chloro-1,1-bis-(4-chlorophenyl)-2,2,2-trichloroethane

[a] Concentrations of dicofol technical represent the technical material (88.5% a.i. [hydrolysable chlorine]; 78.5% *p,p′*-dicofol and 13.11% *o,p′*-dicofol [HPLC]); all other concentrations represent active ingredient concentrations.

liver weight, body weight, liver to body weight ratio and liver microsomal protein concentration were measured. The activity of MFO was assessed in liver microsomes by *p*-nitroanisole *O*-demethylation (PNA), aminopyrine *N*-demethylation (AP) and aniline hydroxylation (AH).

Administration of 8, 25, 80, 250 or 800 ppm dicofol technical to male B6C3F1 mice significantly increased liver weight by 17% and 43% at 250 and 800 ppm, respectively, and liver to body weight ratio by 34% and 79% at 250 and 800 ppm, respectively. Body weight was decreased at 80 ppm and above from 8.4% to 20%. Administration of 6, 20, 63, 195 or 625 ppm a.i. *p,p′*-dicofol significantly increased liver weight by 39%, increased liver to body weight ratio by 60% and decreased body weight by 13% at the highest dose of 625 ppm. The results are shown in Table 38.

The effects of the tested compounds on relative liver microsomal protein concentration and relative liver MFO activity are shown in Table 39. Dicofol technical significantly increased liver microsomal protein concentration by 44% and 52% at 250 and 800 ppm, respectively, and significantly increased liver MFO activity by 1.5- to 3.5-fold, as measured by PNA, AP and AH. *p,p′*-Dicofol significantly increased liver MFO activity by 29–90% at 63 ppm and above, as measured by AH, and by 106–120% at 195 ppm and above, as measured by AP.

Administration of 37.5 ppm a.i. *p,p′*-ER-8, *o,p′*-ER-8, *p,p′*-DDE or *o,p′*-DDE, 20, 63 or 175 ppm a.i. *p,p′*-DCBP or 195 ppm a.i. *o,p′*-DCBP to male B6C3F1 mice in the diet for 2 weeks had no effect on liver weight, body weight, liver to body weight ratio, liver microsomal protein concentration or liver MFO activity.

Based on MFO activity, the minimum effect level for dicofol technical when administered for 2 weeks in the diet to male B6C3F1 mice was 250 ppm, and the NOAEL was 80 ppm. Overall, the minimum effect level for dicofol technical was 80 ppm, and the NOAEL was 25 ppm (i.e. effect on terminal body weight).

Based on MFO activity (and overall), the minimum effect level for *p,p′*-dicofol when administered for 2 weeks in the diet to male B6C3F1 mice was 63 ppm, and the NOAEL was 20 ppm.

Table 38. Effect of dicofol technical, p,p′-dicofol, o,p′-dicofol and technical impurities on liver weight, body weight and liver to body weight ratio in male B6C3F1 mice[a]

Group	Test substance	Concentration of test substance in diet (ppm)[b]	Liver weight (g)	Body weight (g)[c]	Liver to body weight ratio (%)
1A	Vehicle	0	1.34 ± 0.03	25.0 ± 0.4	5.36 ± 0.12
1B	Dicofol technical	8	1.43 ± 0.05	25.5 ± 0.9	5.61 ± 0.11
1C	Dicofol technical	25	1.40 ± 0.07	24.8 ± 1.1	5.63 ± 0.15
1D	Dicofol technical	80	1.36 ± 0.13	22.9 ± 0.8*	5.96 ± 0.51
1E	Dicofol technical	250	1.57 ± 0.03*	22.0 ± 1.9*	7.18 ± 0.71*
1F	Dicofol technical	800	1.91 ± 0.24*	19.9 ± 0.9*	9.60 ± 0.88*
2A	Vehicle	0	1.60 ± 0.00	24.7 ± 1.2	6.48 ± 0.60
2B	*p,p′*-Dicofol	6	1.43 ± 0.20	24.3 ± 0.9	5.88 ± 0.87
2C	*p,p′*-Dicofol	20	1.48 ± 0.06	24.6 ± 0.3	5.99 ± 0.30
2D	*p,p′*-Dicofol	63	1.60 ± 0.11	25.5 ± 0.8	6.29 ± 0.26
2E	*p,p′*-Dicofol	195	1.57 ± 0.16	23.8 ± 1.7	6.60 ± 0.41
2F	*p,p′*-Dicofol	625	2.23 ± 0.20*	21.5 ± 1.2*	10.39 ± 0.45*
3A	Vehicle	0	1.39 ± 0.20	24.4 ± 1.6	5.73 ± 1.07
3B	*o,p′*-Dicofol	37.5	1.55 ± 0.30	24.2 ± 1.4	6.39 ± 1.17
3C	*p,p′*-ER-8	37.5	1.56 ± 0.05	25.6 ± 1.5	6.12 ± 0.47
3D	*o,p′*-ER-8	37.5	1.47 ± 0.15	23.8 ± 1.2	6.19 ± 0.44
3E	*p,p′*-DDE	37.5	1.56 ± 0.06	24.6 ± 1.1	6.36 ± 0.23
3F	*o,p′*-DDE	37.5	1.53 ± 0.06	24.4 ± 1.6	6.27 ± 0.37
4A	Vehicle	0	1.49 ± 0.27	22.5 ± 2.0	6.61 ± 0.95
4B	*o,p′*-Dicofol	195	1.48 ± 0.24	21.9 ± 2.0	6.74 ± 0.67
4C	*p,p′*-DCBP	20	1.27 ± 0.14	22.8 ± 1.7	5.60 ± 0.70
4D	*p,p′*-DCBP	63	1.36 ± 0.06	22.8 ± 1.3	5.98 ± 0.28
4E	*p,p′*-DCBP	175	1.24 ± 0.10	23.0 ± 1.7	5.39 ± 0.52
4F	*o,p′*-DCBP	195	1.59 ± 0.20	24.2 ± 1.6	6.50 ± 0.61

From Steigerwalt, Deckert & Longacre (1984a)
* Significantly different ($P < 0.05$) from respective control group
[a] Values represent mean ± standard deviation for three or four mice per group.
[b] Test substance was administered for 2 weeks in the diet.
[c] At termination.

The results of this study indicated that *p,p′*-dicofol was responsible for a large majority, but not all, of the induction of liver MFO activity observed in male B6C3F1 mice after treatment with dicofol technical (Steigerwalt, Deckert & Longacre, 1984a; reported in Annex 1, reference *67*).

On the basis of these data, it is not possible to conclude on differences in MFO activity between *p,p′*-dicofol and *o,p′*-dicofol.

In a published study carried out in male Wistar rats treated intraperitoneally with dicofol at a dose of 1, 10 or 25 mg/kg bw for 4 days, the ability of the compound to induce cytochrome P450 enzyme activity was investigated. The results are shown in Table 40.

Treatment with dicofol at 1 mg/kg bw induced a trend towards increases in the levels of the aforementioned enzyme components of the monooxygenase system, P450-dependent monooxygenases and antioxidant enzymes; however, the increases were not statistically significant. Dicofol at 10

Table 39. Effect of dicofol technical, p,p′-dicofol, o,p′-dicofol and technical impurities on relative liver microsomal protein concentration and relative liver mixed function oxidase activity in male B6C3F1 mice[a]

Group	Test substance	Concentration of test substance in diet (ppm)[b]	Relative microsomal protein (mg/g liver)[c]	Relative MFO activity (per mg microsomal protein)[d]			Relative MFO activity (per g liver)[e]		
				PNA[f]	AP[g]	AH[h]	PNA[f]	AP[g]	AH[h]
1A	Vehicle	0	100 ± 0	100 ± 1	100 ± 3	100 ± 8	100 ± 1	100 ± 3	100 ± 8
1B	Dicofol technical	8	114 ± 20	91 ± 0	80 ± 1	88 ± 1	103 ± 18	92 ± 16	100 ± 10
1C	Dicofol technical	25	110 ± 5	92 ± 2	95 ± 10	80 ± 16	102 ± 1	104 ± 6	90 ± 13
1D	Dicofol technical	80	111 ± 7	93 ± 3	106 ± 11	102 ± 9	104 ± 9	118 ± 19	114 ± 17
1E	Dicofol technical	250	144 ± 0*	106 ± 8	158 ± 18*	151 ± 31	152 ± 13*	227 ± 25*	220 ± 45*
1F	Dicofol technical	800	152 ± 8*	84 ± 6*	242 ± 13*	158 ± 7*	128 ± 16	368 ± 1*	243 ± 24*
2A	Vehicle	0	100 ± 1	100 ± 1	100 ± 1	100 ± 8	100 ± 1	100 ± 4	100 ± 10
2B	*p,p′*-Dicofol	6	102 ± 11	92 ± 6	88 ± 2	98 ± 9	93 ± 3	90 ± 6	100 ± 0
2C	*p,p′*-Dicofol	20	116 ± 1	91 ± 0	92 ± 16	104 ± 4	106 ± 2	106 ± 19	122 ± 4
2D	*p,p′*-Dicofol	63	120 ± 5	94 ± 4	104 ± 1	106 ± 4	113 ± 8	124 ± 5	129 ± 0*
2E	*p,p′*-Dicofol	195	112 ± 13	89 ± 11	114 ± 18	118 ± 4	98 ± 1	129 ± 34	134 ± 12*
2F	*p,p′*-Dicofol	625	119 ± 3*	61 ± 3*	174 ± 13*	128 ± 8*	72 ± 2*	206 ± 20*	154 ± 13*
3A	Vehicle	0	100 ± 24	100 ± 10	100 ± 8	100 ± 13	100 ± 14	100 ± 32	100 ± 11
3B	*o,p′*-Dicofol	37.5	94 ± 56	104 ± 16	80 ± 22	83 ± 21	94 ± 44	81 ± 66	73 ± 27
3C	*p,p′*-ER-8	37.5	96 ± 37	110 ± 25	131 ± 0	126 ± 19	103 ± 18	126 ± 47	119 ± 31
3D	*o,p′*-ER-8	37.5	106 ± 15	118 ± 30	150 ± 36	110 ± 34	124 ± 15	156 ± 16	115 ± 21
3E	*p,p′*-DDE	37.5	128 ± 4	84 ± 1	120 ± 13	72 ± 7	110 ± 4	152 ± 22	92 ± 11
3F	*o,p′*-DDE	37.5	132 ± 9	90 ± 8	106 ± 13	86 ± 12	121 ± 1	140 ± 8	116 ± 23
4A	Vehicle	0	100 ± 8	100 ± 4	100 ± 4	100 ± 10	100 ± 4	100 ± 11	100 ± 20
4B	*o,p′*-Dicofol	195	137 ± 0	110 ± 7	160 ± 23*	139 ± 10	150 ± 9*	220 ± 31	190 ± 15*
4C	*p,p′*-DCBP	20	96 ± 4	95 ± 4	102 ± 6	127 ± 4	92 ± 6	98 ± 9	122 ± 11
4D	*p,p′*-DCBP	63	76 ± 22	114 ± 1	100 ± 32	154 ± 35	88 ± 25	80 ± 47	112 ± 8
4E	*p,p′*-DCBP	175	102 ± 6	96 ± 11	104 ± 16	122 ± 8	98 ± 4	106 ± 9	125 ± 0
4F	*o,p′*-DCBP	195	92 ± 20	97 ± 17	98 ± 7	152 ± 32	87 ± 4	90 ± 13	136 ± 0

From Steigerwalt, Deckert & Longacre (1984a)

* Significantly different ($P < 0.05$) from respective control group

[a] Values represent the mean ± standard deviation for two samples per group; each sample was prepared from liver postmitochondrial supernatant fraction from one or two mice.

[b] Test substance were administered for 2 weeks in the diet.

[c] Milligrams of microsomal protein per gram of liver relative to respective control group.

[d] MFO activity (mmol product per milligram microsomal protein) relative to respective control group.

[e] MFO activity (mmol product per gram liver) relative to respective control group.

[f] *p*-Nitroanisole *O*-demethylation activity (relative to respective control group).

[g] Aminopyrine *N*-demethylation activity (relative to respective control group).

[h] Aniline hydroxylation activity (relative to respective control group).

Table 40. Effects of treatment with dicofol on cytochrome P450–dependent monooxygenases and conjugation and antioxidant enzymes in rat liver

	Dose (mg/kg bw)			
	0 (control)	1	10	25
Body weight (g)	207.5 ± 5.5	206.3 ± 11.3	208.7 ± 6.1	192.7 ± 5.7
Liver weight to body weight ratio (g/g × 100)	4.480 ± 0.167	4.617 ± 0.106	5.039 ± 0.077* (+13%)	5.180 ± 0.133* (+16%)
Microsomal protein (mg/g liver)	23.73 ± 1.11	23.32 ± 2.10	26.52 ± 1.39	29.40 ± 2.67
Cytochrome P450 (nmol/mg protein)	0.481 ± 0.037	0.508 ± 0.018	1.024 ± 0.083* (+112%)	1.407 ± 0.269* (+192%)
NADPH-cytochrome *c* reductase (nmol cytochrome *c*/min/mg protein)	146.8 ± 8.7	161.5 ± 5.0	176.4 ± 3.6* (+20%)	219.7 ± 11.0* (+ 50%)
Cytochrome b_5 (nmol/mg protein)	0.361 ± 0.017	0.413 ± 0.009	0.473 ± 0.021* (+31%)	0.488 ± 0.026* (+35%)
7-Ethoxyresorufin *O*-deethylase (pmol RF/min/mg protein)	28.2 ± 2.5	34.0 ± 2.8	38.6 ± 0.9* (+34%)	44.2 ± 2.5* (+57%)
7-Ethoxycoumarin *O*-deethylase (nmol HC/min/mg protein)	0.704 ± 0.051	0.872 ± 0.063	1.765 ± 0.089* (+150%)	2.038 ± 0.132* (+189%)
Methoxyresorufin *O*-demethylase (pmol RF/min/mg protein)	21.5 ± 2.1	22.7 ± 2.2	22.9 ± 1.7	25.3 ± 0.7
Pentoxyresorufin *O*-dealkylase (pmol RF/min/mg protein)	20.3 ± 1.2	29.4 ± 1.3	272.9 ± 45.0* (+13.4-fold)	319.0 ± 34.2* (+15.7-fold)
Aniline hydroxylase (nmol PAP/min/mg protein)	0.667 ± 0.029	0.700 ± 0.030	0.854 ± 0.043* (+28%)	0.899 ± 0.038* (+35%)
Erythromycin *N*-demethylase (nmol HCHO/min/mg protein)	0.291 ± 0.045	0.320 ± 0.049	0.513 ± 0.058* (+13.4-fold)	0.618 ± 0.033* (+15.7-fold)
Uridine diphosphate glucuronosyltransferase (nmol NG/min/mg protein)	28.9 ± 2.8	30.3 ± 2.9	34.3 ± 2.1	38.7 ± 6.6
Glutathione *S*-transferase (mol CDNB/min/mg protein)	1.003 ± 0.025	1.069 ± 0.058	1.289 ± 0.056* (+29%)	1.566 ± 0.105* (+56%)
Superoxide dismutase (unit/mg protein)	183.0 ± 22.0	201.0 ± 24.1	278.3 ± 26.0* (+52%)	301.5 ± 24.5* (+65%)
Catalase (unit/mg protein)	136.2 ± 9.4	148.0 ± 6.9	170.1 ± 11.5	178.5 ± 16.5
Glutathione peroxidase (nmol NADPH/min/mg protein)	411.3 ± 16.9	424.0 ± 32.3	436.8 ± 12.7	439.3 ± 23.6

From Chan et al. (2009)
CDNB, 1-chloro-2,4-dinitrobenzene; HC, hepatic cytochrome; HCHO, formaldehyde; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NG, naphthyl glucuronide; PAP, *p*-aminophenol; RF, resorufin

and 25 mg/kg bw significantly induced cytochrome P450 (CYP) and cytochrome b_5 contents and the activity of NADPH-cytochrome *c* reductase, 7-ethoxyresorufin *O*-deethylase, pentoxyresorufin *O*-dealkylase, 7-ethoxycoumarin *O*-deethylase, AH and erythromycin *N*-demethylase.

Immunoblot analysis was also performed, and the results showed that dicofol at 10 and 25 mg/kg bw increased protein levels of CYP1A1, CYP2B, CYP2E1 and CYP3A in liver. Reverse transcriptase polymerase chain reaction data indicated that dicofol induced messenger ribonucleic acid (mRNA) expression of liver CYP1A1 (Chan et al., 2009).

3. Observations in humans

In 1979, 78 incidents of dicofol technical (Kelthane) exposure were reported by the USEPA's Pesticide Incident Monitoring System. Fourteen cases involved dicofol alone, and eight of these reported symptoms. One case involved dicofol ingestion (amount unspecified), leading to nausea, dizziness and vomiting. Three cases involved inhalation exposure, resulting in dizziness, weakness and vomiting in two cases and sinus congestion in the third. Two cases involved dermal exposure (amount unspecified), resulting in skin irritation in one case and rash (allergic reaction) in the other (USEPA, 1979; reported in Annex 1, reference *67*).

In a case report, a 12-year-old male was accidentally exposed to dicofol when he fell from a bicycle into a puddle of spilled undiluted dicofol formulation (470 g/l; 189-litre drum). The skin was abraded and clothing contaminated. The patient had initial symptoms of nausea, dizziness, disorientation, confusion, lethargy and headache and demonstrated horizontal nystagmus and impaired balance. These symptoms resolved within 3 weeks. Three weeks after the incident, serum dicofol levels were 1.1 µg/l, and adipose tissue levels were 0.153 µg/kg (analytical methods unspecified). No dicofol was detected in serum 16 weeks after the exposure. Following persistent emotional difficulties, the patient underwent a neuropsychological evaluation 8 months after the exposure, which showed impairment of certain cognitive functions, including auditory attention, immediate memory and ability to selectively inhibit inappropriate responses. A pre-exposure neuropsychological analysis was unavailable for comparison (Lessenger & Riley, 1991; reported in Annex 1, reference *67*).

In a published study, the dicofol metabolite DCBA was measured in the urine of four workers involved in the mixing/loading or application of dicofol to citrus crops for 10 consecutive days. Urine samples were obtained over 4 days beginning 6 days after exposure. Because of previous use of chlorobenzilate, pre-exposure DCBA excretion rates were not zero. Mean daily DCBA excretion was 19–42 µg/day over the exposure period. The variation correlated with the difference in estimated dermal dose (2.7–13 mg/day). The per cent dermal dose excreted as DCBA was estimated to be 0.25%. The half-life for DCBA excretion in the urine was estimated to be 7 days (WHO/FAO, 1996).

In a published epidemiological study carried out by the California Department of Public Health among children born between 1996 and 1998, women in the first 8 weeks of pregnancy who live within 500 m of farm fields sprayed with dicofol and the related organochlorine pesticide endosulfan were found to be several times more likely to give birth to children with autism. Four hundred and sixty-five children with autism spectrum disorder were identified and matched to 6975 liveborn, normal birth weight, term infants as controls. Of 249 unique hypotheses, 4 that described organochlorine pesticide applications—specifically those of dicofol and endosulfan—occurring during the period immediately before and concurrent with central nervous system embryogenesis (clinical weeks 1 through 8) met a priori criteria and were unlikely to be a result of multiple testing. Multivariate a posteriori models compared children of mothers living within 500 m of field sites with the highest non-zero quartile of organochlorine poundage with those of mothers not living near field sites. The suggested odds ratio was 6.1 (95% confidence interval 2.4–15.3). The risk of autism increased with the poundage of organochlorine pesticide applied and decreased with distance from field area. However, these results are highly preliminary due to the small number of women and children involved and lack of evidence from other studies (Roberts et al., 2007).

Comments

Biochemical aspects

Dicofol was almost completely and rapidly absorbed from the gastrointestinal tract within 24 hours following an oral dose in rats. Fat contained the highest concentration of dicofol, followed by adrenals, thyroid, liver and whole blood. After a single oral dose, the maximum tissue levels were reached 24–48 hours post-dosing, and the maximum excretion level was attained between 24 and 96 hours after dosing (half-life of 30 hours). The elimination of dicofol from the body was relatively slow, with greater than 65% of administered radioactivity still present in the carcass after 48 hours. Excretion occurred via faeces and urine, faeces being the main route of elimination. There is some indication of accumulation of dicofol in fat. Although dicofol accounts for most of the radioactivity in fat, it is only a minor component of the radioactivity in urine and faeces, indicating extensive metabolism after mobilization. The main metabolites are DBCH (in males), FW-152 and OH-DCBP (especially in females). Significant conjugation with glycine also occurs.

Toxicological data

The acute oral toxicity (LD_{50}) was 669 mg/kg bw in mice, 578 mg/kg bw in rats, 1810 mg/kg bw in rabbits and greater than 4000 mg/kg bw in dogs. Clinical signs of toxicity include decreased spontaneous motor activity, ataxia, passiveness, somnolence, prostration and occasionally tremors. In rabbits, dicofol was a slight to moderate irritant for skin and eyes. It gave a positive response for skin sensitization in a modified Buehler test in guinea-pigs.

In a single-dose gavage study in rats, the NOAEL was 15 mg/kg bw, based on decreased feed intake and hypertrophy of the adrenal zona fasciculata at 75 mg/kg bw.

The primary effects of dicofol after short- or long-term exposure were body weight reduction associated with decreased feed intake and increased liver weight accompanied by increased hepatic MFO activity and liver hypertrophy in mice, rats and dogs, increased serum ALT and serum alkaline phosphatase activities and hepatocellular necrosis at higher doses. Increases in hepatocyte hypertrophy and liver weight with no other effects were considered to be adaptive and not treated as adverse effects; other histopathological findings in the liver, such as fatty vacuolation and necrosis, were treated as adverse. At high doses, changes in the kidneys, adrenals, heart and testes were also observed in rodents. Reduced serum cortisone levels were seen in dogs, indicating disturbances in adrenocorticoid metabolism.

The short-term effects of dicofol were studied in 90-day feeding studies in mice, rats and dogs and in a 1-year feeding study in dogs.

In a 13-week dietary study in mice, slightly reduced final body weights in both sexes, increased hepatic MFO activity in both sexes and increased absolute and relative liver weights in females (by 20% and 25%) were observed at 125 ppm. At a dose of 250 ppm and above, dicofol induced hepatocellular hypertrophy in both sexes, increased ALT activity in males and females (by 68% and 78%, respectively) and decreased absolute kidney weight in females by 10%. At 500 and 1000 ppm, degenerative changes in the kidney of females, adrenal cortical hypertrophy and hepatocellular necrosis and vacuolation were observed. The NOAEL was 125 ppm (equal to 18 mg/kg bw per day), based on increased ALT activity and other liver effects at 250 ppm (equal to 38 mg/kg bw per day).

In a 13-week feeding study in rats, a dose of 1500 ppm caused death in 5 of 10 male and 8 of 10 female rats. The feed intake and mean body weights were significantly decreased in males and females fed diets containing 500 ppm and above. Clinical signs of scant droppings, soft faeces and/or faeces with mucus were seen in females at 500 ppm. Changes in haematology and clinical chemistry parameters and liver hypertrophy accompanied by increased liver weight were observed at 500 ppm and above. Kidney and adrenal weights were also significantly increased. The incidence and severity

of thyroid follicular cell hypertrophy (minimal to moderate) were increased in males at 10 ppm and above, but the end-point was considered to be of doubtful toxicological significance, as no changes in TSH, T_3 or T_4 were observed in a long-term toxicity study in rats. The NOAEL was 100 ppm (equal to 6.5 mg/kg bw per day), based on the reduction in mean body weights at 500 ppm (equal to 32 mg/kg bw per day) in both sexes.

In a 13-week dietary study in dogs, the highest tested concentration of 1000 ppm caused death in five of six dogs of each sex. Clinical signs of toxicity (laboured breathing, inactivity, dehydration, red-tinged diarrhoea, incoordination and excessive salivation) were observed at 300 ppm and above. ALT activity was significantly increased at 1000 ppm, and serum alkaline phosphatase activity was increased at 300 ppm and above in females. Dicofol at 300 ppm and above caused prolongation of the QT interval. In male dogs fed dietary concentrations of 300 or 1000 ppm, a decrease in spermatogenesis (3/6 and 5/6 males, respectively) and an increase in mean hepatic weights were observed. Gross lesions, as well as microscopic lesions in the liver, testes and heart, were observed at 1000 ppm in both sexes. Dicofol at dietary concentrations of 100 ppm and above caused reduced cortisol response to ACTH. The NOAEL was 10 ppm (equal to 0.29 mg/kg bw per day), based on reduced cortisol response to ACTH challenge at 100 ppm (equal to 3.3 mg/kg bw per day).

In a 52-week dietary study in dogs, adverse findings occurred only at the highest tested concentration of 180 ppm and were more prominent in males than in females. This concentration resulted in increased serum alkaline phosphatase activity and cholesterol levels in males, decreased albumin in both sexes and a significant increase in LDH activity in females at week 39. Relative (to body and brain weight) liver weights were increased in males but were unchanged in females. Baseline cortisol blood levels were normal, but cortisol response to ACTH challenge (20 units of ACTH; cortisol measured 30 and 90 minutes after challenge) was markedly decreased in both sexes at 180 ppm. Minimal to mild hepatocellular hypertrophy was observed in five of six dogs of each sex. The NOAEL was 30 ppm (equal to 0.82 mg/kg bw per day), based on histological and clinical chemistry changes at 180 ppm (equal to 5.4 mg/kg bw per day).

The overall NOAEL for the two dog studies was considered to be 30 ppm (equal to 0.82 mg/kg bw per day), with an overall lowest-observed-adverse-effect level (LOAEL) of 100 ppm (equal to 3.3 mg/kg bw per day).

In a 78-week toxicity and carcinogenicity study in mice, using time-weighted average dietary concentrations of 264 or 528 ppm (equivalent to 40 or 80 mg/kg bw per day) in males and of 122 and 243 ppm (equivalent to 18 and 36 mg/kg bw per day) in females, an increased number of liver adenomas and combined adenomas and carcinomas was observed in males at 264 and 528 ppm. The incidence of hepatocellular carcinomas was increased at both doses compared with controls, but there was no pairwise or trend significance. No treatment-related tumours were observed in female mice at doses up to 243 ppm. Survival in male and female mice was not affected in this study. There was a decrease in the body weights of high-dose females. The body weights of male mice were not affected. The NOAEL in female mice was 122 ppm (equivalent to 18 mg/kg bw per day), based on decreased body weight at 243 ppm (equivalent to 36 mg/kg bw per day). A NOAEL in male mice was not observed. The LOAEL in male mice was 264 ppm (equivalent to 40 mg/kg bw per day), based on the increase in hepatocellular adenomas.

In a 78-week carcinogenicity study in rats, using time-weighted average dietary concentrations of 470 or 940 ppm (equivalent to 24 and 47 mg/kg bw per day) for males and of 380 or 760 ppm (equivalent to 19 and 38 mg/kg bw per day) for females, no treatment-related clinical signs were observed, and no neoplastic or non-neoplastic lesions were associated with dicofol treatment. The NOAEL in this study was 760 ppm (equivalent to 38 mg/kg bw per day), the highest dose tested.

In a 2-year study in rats, terminal body weights were decreased in both males and females at 250 ppm. Both male and female rats fed with dietary concentrations of dicofol of 50 and 250 ppm had decreased feed consumption, increased MFO activity and increased relative liver weight. Treatment-

related microscopic changes in the liver, which included hepatocellular necrosis, and vacuolation in adrenal glands were also observed in animals of both sexes that received dicofol at 50 and 250 ppm. The NOAEL was 5 ppm (equal to 0.22 mg/kg bw per day), based on histopathological changes in the liver and adrenal gland at 50 ppm (equal to 2.2 mg/kg bw per day). No treatment-related tumours were observed in this study.

The Meeting concluded that dicofol causes liver tumours in male mice at doses associated with significant enzyme induction and liver hypertrophy, which are anticipated to exhibit a threshold response.

Dicofol gave a negative response in an adequate range of in vitro genotoxicity and in vivo chromosomal aberration tests.

The Meeting concluded that dicofol is unlikely to be genotoxic.

On the basis of the absence of genotoxicity, the absence of carcinogenic effects in rats and the expectation that the adenomas present in mice will exhibit a threshold, the Meeting concluded that dicofol is unlikely to pose a carcinogenic risk to humans at anticipated dietary exposure levels.

In a two-generation reproduction study in rats, F_0 females receiving 125 or 250 ppm showed reduced body weight gain and feed consumption. Treatment-related vacuolation was observed in the liver, ovaries and adrenal glands of F_0 and F_1 parental rats. Offspring toxicity was observed in F_1 and F_2 pups at 125 and 250 ppm. Viability was reduced in F_1 pups at 125 ppm and statistically significantly at 250 ppm and in F_2 pups statistically significantly at 125 and 250 ppm.

The NOAEL for reproductive toxicity was 25 ppm (equal to 2.1 mg/kg bw per day), based on decreased viability at 125 ppm (equal to 10 mg/kg bw per day). The NOAEL for parental toxicity was 5 ppm (equal to 0.5 mg/kg bw per day), based on histopathological changes in the liver and ovaries at 25 ppm (equal to 2.1 mg/kg bw per day). The NOAEL for offspring toxicity was 25 ppm (equal to 2.1 mg/kg bw per day), based on decreased viability index and increased number of litters with all offspring dying at 125 ppm (equal to 10 mg/kg bw per day).

In an enhanced one-generation study on reproduction in rats, a transient decrease in body weights, organ weight changes (liver, kidney and ovary) and histopathological changes in the liver were observed at the highest dose tested. No treatment-related effects on sperm parameters or other reproductive organs (estrous cycle, sexual maturation) were observed at doses up to 125 ppm. The NOAEL for parental toxicity was 25 ppm (equal to 1.7 mg/kg bw per day), based on the transient decrease in body weight, organ weight changes and histopathological findings in the liver seen at 125 ppm (equal to 8.7 mg/kg bw per day). The NOAEL for reproductive and offspring toxicity was 125 ppm (equal to 8.7 mg/kg bw per day), the highest dose tested.

In a developmental toxicity study in rats, the maternal toxicity NOAEL was 2.5 mg/kg bw per day, based on a statistically significant reduction in maternal body weight gain, decreased feed consumption and feed efficiency and increased relative liver weight at 25 mg/kg bw per day. The increased incidence of salivation observed at 2.5 and 25 mg/kg bw was not confirmed in the range-finding developmental study or an acute neurotoxicity study. Therefore, this was not considered to be related to treatment. The NOAEL for embryotoxicity and teratogenicity was 25 mg/kg bw per day, the highest dose tested.

In a study of developmental toxicity in rabbits, the NOAEL was 4 mg/kg bw per day, based on abnormal faeces (dried, soft or liquid), decreased feed consumption, maternal weight loss, a significant increase in the incidences of abortion (4/19, control 1/18) and increased relative liver weight at 40 mg/kg bw per day. No treatment-related effects on fetal viability, average fetal body weights or external, soft tissue or skeletal examination were observed at doses up to 40 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 40 mg/kg bw per day, the highest dose tested.

The Meeting concluded that dicofol was not teratogenic.

In an acute neurotoxicity study, the NOAEL was 15 mg/kg bw per day. At 75 and 350 mg/kg bw per day, reduced body weights and feed consumption were observed in both male and female rats; an increased number of rats in these groups had urine-stained or faeces-stained fur. Male and female rats given the 350 mg/kg bw per day dose had ataxia, other signs of sensorimotor dysfunction and decreased motor activity within the week after treatment. However, these effects were not evident 2 weeks after administration. The neurohistological evaluation of rats in the 350 mg/kg bw per day dose group did not reveal any pathology related to the test substance.

The NOAEL from a 90-day neurotoxicity study was 5 ppm (equal to 0.2 mg/kg bw per day), based on affected parameters in the FOB, altered absolute and relative organ weights and reduced body weight, feed consumption values and motor activity at 100 ppm (equal to 6.7 mg/kg bw per day). The neurohistological examination of the 500 ppm group rats did not reveal any pathology related to the test substance.

Reports of cases of acute poisoning indicate that dicofol causes signs and symptoms such as nausea, dizziness, vomiting, confusion and lethargy. The symptoms resolved within 3 weeks. Epidemiological studies on children exposed to dicofol, among other chemicals, were inconclusive.

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

Toxicological evaluation

After evaluation of new information and re-evaluation of previous data, the Meeting confirmed the ADI of 0–0.002 mg/kg bw derived from the NOAEL in the 2-year toxicity and carcinogenicity study in rats of 0.22 mg/kg bw per day, based on histopathological changes in the liver and adrenal gland. A safety factor of 100 was applied. The ADI is supported by the NOAEL of 0.2 mg/kg bw per day from the 90-day neurotoxicity study in rats. There is a margin of 20 000 between the maximum ADI and the LOAEL for liver adenomas in the male mouse.

An acute reference dose (ARfD) of 0.2 mg/kg bw was established on the basis of the NOAEL of 15 mg/kg bw in the acute neurotoxicity study in rats, based on decreased body weight and decreased feed intake at 75 mg/kg bw. This ARfD was supported by the NOAEL of 15 mg/kg bw in a single-dose oral toxicity study in rats, based on decreased feed intake and hypertrophy of adrenal zona fasciculata at 75 mg/kg bw. Although these effects were mild, they were observed in two studies, and therefore 75 mg/kg bw was considered a marginal LOAEL. A safety factor of 100 was applied.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Thirteen-week study of toxicity[a]	Toxicity	125 ppm, equal to 18 mg/kg bw per day	250 ppm, equal to 38 mg/kg bw per day
	Seventy-eight-week study of toxicity and carcinogenicity[a]	Toxicity	122 ppm, equivalent to 18 mg/kg bw per day	243 ppm, equivalent to 36 mg/kg bw per day
		Carcinogenicity[b]	—	264 ppm, equivalent to 40 mg/kg bw per day[c]
Rat	Thirteen-week study of toxicity[a]	Toxicity	100 ppm, equal to 6.5 mg/kg bw per day	500 ppm, equal to 32 mg/kg bw per day
	Two-year studies of toxicity and carcinogenicity[a,d]	Toxicity	5 ppm, equal to 0.22 mg/kg bw per day	50 ppm, equal to 2.2 mg/kg bw per day
		Carcinogenicity	250 ppm, equal to 14 mg/kg bw per day[e]	—

Species	Study	Effect	NOAEL	LOAEL
	Single oral dose toxicity study[f]	Toxicity	15 mg/kg bw per day	75 mg/kg bw per day
	Two-generation study of reproductive toxicity[a]	Parental toxicity	5 ppm, equal to 0.5 mg/kg bw per day	25 ppm, equal to 2.1 mg/kg bw per day
		Reproductive toxicity	25 ppm, equal to 2.1 mg/kg bw per day	125, equal to 10 mg/kg bw per day
		Offspring toxicity	25 ppm, equal to 2.1 mg/kg bw per day	125 ppm equal to 10 mg/kg bw per day
	One-generation study of reproduction[a]	Parental toxicity	25 ppm, equal to 1.7 mg/kg bw per day day	125 ppm, equal to 8.7 mg/kg bw per day
		Reproductive toxicity	125 ppm, equal to 8.7 mg/kg bw per day[e]	—
		Offspring toxicity	125 ppm, equal to 8.7 mg/kg bw per day[e]	—
	Developmental toxicity[f]	Maternal toxicity	2.5 mg/kg bw per day	25 mg/kg bw per day
		Embryo and fetal toxicity	25 mg/kg bw per day[e]	—
	Acute neurotoxicity[f]	Neurotoxicity	15 mg/kg bw per day	75 mg/kg bw per day
	Ninety-day neurotoxicity study[a]	Neurotoxicity	5 ppm, equivalent to 0.2 mg/kg bw per day	100 ppm, equivalent to 6.7 mg/kg bw per day
Rabbit	Developmental toxicity[f]	Maternal toxicity	4 mg/kg bw per day	40 mg/kg bw per day
		Embryo and fetal toxicity	40 mg/kg bw per day[e]	—
Dog	Thirteen-week and 1-year studies of toxicity[a,d]	Toxicity	30 ppm, equal to 0.82 mg/kg bw per day	100 ppm, equal to 3.3 mg/kg bw per day

[a] Dietary administration.
[b] Male liver adenomas only.
[c] Lowest dose tested.
[d] Two or more studies combined.
[e] Highest dose tested.
[f] Gavage administration.

Estimate of acceptable daily intake for humans

0–0.002 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 exposure

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

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Extensively absorbed from the gastrointestinal tract within 24 h
Distribution	Adipose tissue > adrenal gland > thyroid > liver > whole blood
Potential for accumulation	Slightly, in fat
Rate and extent of excretion	Majority excreted within 96 h, primarily in faeces
Metabolism in animals	Metabolism involved dechlorination and oxidation of the ethanol moiety and hydroxylation of the aromatic rings
Toxicologically significant compounds (animals, plants and the environment)	Dicofol
Acute toxicity	
Rat, LD_{50}, oral	578 mg/kg bw (purity 94–96%)
Rat, LD_{50}, dermal	> 5000 mg/kg bw
Rat, LC_{50}, inhalation	> 5.0 mg/l
Rabbit, skin irritation	Slight to moderately irritating
Rabbit, eye irritation	Slight to moderately irritating
Guinea-pig, skin sensitization (Buehler test)	Slight to moderate senzitizer
Short-term studies of toxicity	
Target/critical effect	Decreased body weight; reduced cortisol response (dogs)
Lowest relevant oral NOAEL	0.82 mg/kg bw per day (dogs)
Lowest relevant dermal NOAEL	3 mg/kg bw per day (90-day study in dogs)
Lowest relevant inhalation NOAEC	Not available
Genotoxicity	
	Not genotoxic
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Decreased body weight, hepatocellular necrosis, increased ALT and alkaline phosphatase
Lowest relevant NOAEL	0.22 mg/kg bw per day (2-year toxicity/carcinogenicity study in rats)
Carcinogenicity	Unlikely to pose a carcinogenic risk to humans at anticipated dietary exposure levels
Reproductive toxicity	
Reproduction target/critical effect	Decreased viability index (rats)
Lowest relevant reproductive NOAEL	2.1 mg/kg bw per day
Developmental target/critical effect	None
Lowest relevant developmental NOAEL	40 mg/kg bw per day (highest dose tested)
Neurotoxicity/delayed neurotoxicity	
Acute neurotoxicity target/critical effect	Ataxia, decreased motor activity at systemically toxic dose
Lowest relevant acute neurotoxicity NOAEL	15 mg/kg bw
Subchronic neurotoxicity target/critical effect	Decreased motor activity at systemically toxic doses
Lowest relevant subchronic neurotoxicity NOAEL	0.2 mg/kg bw per day (90-day neurotoxicity study)

Other toxicological studies	
	No data
Medical data	
	Reversible neurological effects and nonspecific symptoms after acute poisoning

Summary

	Value	Study	Safety factor
ADI	0–0.002 mg/kg bw	Chronic toxicity/carcinogenicity study in rats supported by the 90-day neurotoxicity study in rats	100
ARfD	0.2 mg/kg bw	Acute neurotoxicity study in rats	100

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EMAMECTIN BENZOATE

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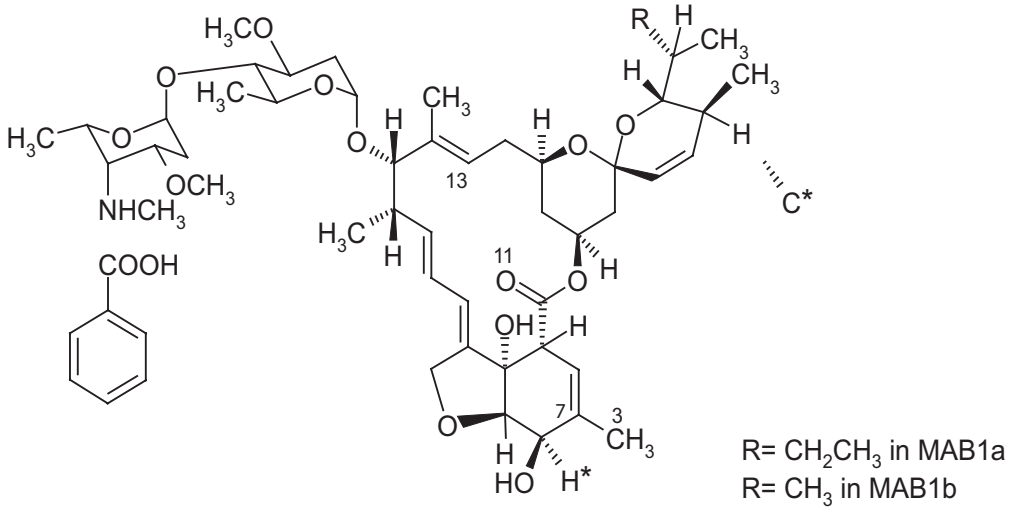
Explanation

Emamectin benzoate is the International Organization for Standardization (ISO)–approved name for (4″*R*)-4″-deoxy-4″-(methylamino)avermectin B1 benzoate (MAB1 benzoate), with Chemical Abstracts Service No. 155569-91-8. It is a macrocyclic lactone insecticide derived from the avermectin series of natural products. It is a mixture of at least 90% (4″*R*)-4″-deoxy-4″-(methylamino)-avermectin B1a benzoate (MAB1a or emamectin B1a benzoate) and at most 10% (4″*R*)-4″-deoxy-4″-(methylamino)avermectin B1b benzoate (MAB1b or emamectin B1b benzoate) salts. Emamectin is structurally similar to abamectin and ivermectin.

Figure 1. Chemical structure of emamectin benzoate. The positions of the radiolabel ^{3}H (H*) and ^{14}C (C*) used in the toxicokinetics studies are shown. Extra ^{14}C labels were used in the Mushtaq (1993) study at the 3, 7, 11 and 13 carbon atoms.



Emamectin was originally developed as the hydrochloride salt MK 243 (L-656,748-010V), but the commercial product was subsequently changed to the benzoate salt MK 244 (L-656,748-038W) and benzoate hydrate (L-656,748-052S) because of superior storage and handling characteristics. Emamectin is being evaluated for the first time by the Joint FAO/WHO 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

The structure of emamectin is shown in Figure 1.

1. Biochemical aspects

1.1 Absorption, distribution and excretion

Mice

Several avermectins have been shown to interact with the adenosine triphosphate–binding cassette (ABC) transporter p-glycoprotein. The ABC transporter p-glycoprotein is widely distributed in tissues, but importantly, in the present context, it is expressed in capillary endothelial cells constituting the blood–brain barrier. It is involved in the transmembrane transport of various molecules, and it functions to remove toxic substances from the brain. Gledhill (2008) performed a study to determine the role of p-glycoprotein in the toxicokinetics of emamectin. For this, mutant CF-1 mice were used that are deficient in expression of mdr1a p-glycoprotein. The study was performed according to Organisation for Economic Co-operation and Development (OECD) Test Guideline 417. Three pesticides were tested: emamectin, abamectin and ivermectin. Only the methods and results for emamectin are described here. CF-1 wild-type (44 females) and mutant (48 females) mice were orally administered emamectin (unlabelled: batch reference 454893, purity 95.5%; ^{3}H-labelled MAB1a: batch reference TRQ11421, purity > 96%) at a dose of 0.1 mg/kg body weight (bw). The position

of the ^{3}H label is indicated by the asterisk in Figure 1. Emamectin was tested in a preliminary study (four mutant female mice) to assess the suitability of dosing with 0.1 mg/kg bw in relation to toxicity. In the main study, mice were killed at 1, 2, 4, 6, 8, 12, 24, 48, 72 and 96 hours after dosing (4–5 mice per time point), and blood and bile samples were taken at each time point. Urine and faeces were collected at 0–12, 12–24, 24–48, 48–72 and 72–96 hours and used to measure radioactivity levels. The brains were collected at each termination for further radiochemical analyses by liquid scintillation counting (LSC). All mice were observed daily for abnormalities.

No clinical abnormalities were observed in wild-type or mutant mice after administration of emamectin in either the preliminary or the main study. One exception is a wild-type mouse (main study) that showed lethargy, loss of hindlimb function and shallow breathing. It also had bleeding in the brain that was more marked than with the other mice.

In wild-type mice, absorption of radiolabel resulted in maximum concentrations in the blood and plasma (C_{max}) of 0.013 and 0.026 µg equivalent (eq) per gram, respectively, at 8 hours (Table 1). The concentration declined steadily after that until 96 hours, at which time only very low concentrations were observed (≤ 0.0005 µg eq/g). In mutant mice, C_{max} values were about one third to one half higher and attained in 12 hours. Elimination from blood was slower in the mutant mice, as seen by a 2-fold higher half-life and a 3- to 4-fold higher area under the curve (AUC) compared with wild-type mice. In brain tissue, the C_{max} value for wild-type mice was very low (0.002 µg eq/g) and was reached at 12 hours, whereas the C_{max} for mutant mice was about 80-fold higher and reached only after 24 hours. Elimination was complete by 96 hours in wild-type mice (Table 1), but in mutant mice, the concentration of radioactivity remained at 0.034 µg eq/g brain at this time. The AUC for the brain was 161 times higher in mutant mice than in wild-type mice. Concentrations were 5–10 times higher in the brain than in blood in mutant mice, whereas in wild-type mice, the concentrations in the brain were up to 6 times lower than in blood.

Emamectin-derived radioactivity was eliminated mainly via faeces in wild-type mice. By 24, 48, 72 and 96 hours, the proportions of the administered dose recovered in the faeces were 69% (n = 33), 81% (n = 3), 88% (n = 2) and 88% (n = 1), respectively. In urine, only 0.4–0.5% of the dose was excreted over the period 0–96 hours. Low levels of radioactivity were measured in bile (per cent recovery not determined). In wild-type mice, total recovery of radioactivity via faeces, urine and cage wash averaged 88% by 96 hours. Elimination via faeces was also the main excretion route of emamectin in mutant mice, but with lower percentages compared with wild-type mice. The recoveries in faeces of mutant mice were 25% (n = 3), 42% (n = 3), 56% (n = 2) and 67% (n = 1) over the periods 0–24, 0–48, 0–72 and 0–96 hours, respectively. Excretion in urine of mutant mice was 1.6–1.9% over 96 hours. Radioactivity levels in bile from mutant mice were low (Gledhill, 2008).

Rats

Groups of male and female Crl:CD(SD)BR rats were treated with single or repeated oral (gavage) doses of ^{3}H-labelled MAB1a (L-683,825-005J003 and L-683,825-005J004; radiochemical purity 97.6% and 98.2%, respectively), diluted with non-labelled emamectin benzoate (L-656,748-052S 005, purity 89.1% MAB1a and 6.3% MAB1b), suspended in propylene glycol/saline (50:50 volume per volume [v/v]). The position of the label is indicated by the asterisk in Figure 1. An overview of the study design is presented in Table 2.

Rats from groups E and F were cannulated in the bile duct 1 day before dosing. Rats from groups G and H received daily administrations of emamectin for 14 days. All samples were analysed for radioactivity by LSC. Metabolites were analysed in urine, faeces, bile, plasma and tissues (see section 1.2).

During the whole study, no major signs of toxicity were observed in the rats. The results of the kinetics experiments are presented in Table 3. Concentration profiles of MAB1a in whole blood and plasma were similar. Maximum concentrations were reached 5–7 hours after administration of a single low dose and 11–15 hours after a single high dose. Comparison of the C_{max} values in the low- and

Table 1. Toxicokinetics of ^{3}H-labelled emamectin in blood, plasma and brain from wild-type and mutant mice

Parameters	Blood		Plasma		Brain	
	Wild-type	Mutant	Wild-type	Mutant	Wild-type	Mutant
C_{max} (µg eq/g)	0.013	0.019	0.026	0.034	0.002	0.167
T_{max} (h)	8	12	8	12	12	24
$t_{½}$ (h)	18.6	37.6	19.5	NR	30.3	31.4
AUC (µg·h/g)	0.23	0.89	0.446	1.408	0.052	8.405

From Gledhill (2008)
AUC, area under the curve; C_{max}, maximum concentration; NR, not reported; $t_{½}$, half-life; T_{max}, time to reach C_{max}

Table 2. Overview of study design

Test group	Number and sex	Dose (mg/kg bw per day)	Comments	Sampling regime
A	6M/6F	0.5 (single)	Kinetics	Blood samples (pre-dose and 1, 2, 4, 6, 12, 24, 48, 72, 96, 120, 144, 168 h after dosing)
B	6M/6F	20 (single)	Kinetics	Blood samples (pre-dose and 1, 2, 4, 6, 12, 24, 48, 72, 96, 120, 144, 168 h after dosing)
C	12M/12F	0.5 (single)	Tissue distribution/ excretion	3M/3F at each time point, range of tissues and organs after 3, 6, 24, 168 h; collection of urine and faeces at each time point and every 24 h
D	12M/12F	20 (single)	Tissue distribution/ excretion	3M/3F at each time point, range of tissues and organs after 3, 12, 60, 168 h; collection of urine and faeces at each time point and every 24 h
E	4M/4F	0.5 (single)	Biliary excretion	Bile was collected pre-dose and 1, 2, 3, 4, 6, 10, 12, 24, 48 h after dosing; urine and faeces were collected pre-dose and 6, 12, 24, 48 h after dosing
F	4M/4F	20 (single)	Biliary excretion	Bile was collected pre-dose and 1, 2, 3, 4, 6, 10, 12, 24, 48 h after dosing; urine and faeces were collected pre-dose and 6, 12, 24, 48 h after dosing
G	6M/6F	0.5 (repeated, 14 days)	Kinetics	Blood samples (pre-dose and 23, 95, 167, 239, 312, 313, 314, 316, 318, 324, 336, 360, 384, 408, 432, 456, 480 h after the first dose)
H	12M/12F	0.5 (repeated, 14 days)	Tissue distribution/ excretion	3M/3F at each time point, range of tissues and organs 1, 6, 42, 168 h after the final dose; collection of urine and faeces every 24 h and 1, 6 h after the final dose

From Powles & Thornley (1995)
F, female; M, male

high-dose groups indicates dose proportionality. In the rats that received 14 repeated oral doses, the C_{max} was reached 6 hours after the last dose. In the single low- and high-dose studies, as well as the repeated low-dose study, oral absorption was lower in females than in males, as shown by the lower C_{max} and AUC levels in females compared with males. Half-lives were higher following a single high dose and repeated dose compared with a single low dose. Females showed shorter elimination times than males upon low-dose treatment. Repeated-dose administration resulted in a steady-state situation at about the seventh dose.

Following administration of MAB1a, radioactivity was observed in all tissues (including bone marrow), irrespective of the dosing regime. Distribution to the tissues was similar in males and females. The highest levels of MAB1a radioactivity in tissues were found in small intestine, caecum,

Table 3. Toxicokinetic data for blood and plasma after a single or repeated oral dose administration of ^{3}H-labelled MAB1a

	Group A (single dose, 0.5 mg/kg bw)		Group B (single dose, 20 mg/kg bw)		Group G (repeated dose, 0.5 mg/kg bw)	
	Males	Females	Males	Females	Males	Females
Blood						
C_{max} (µg eq/ml)	0.029	0.025	1.26	0.91	0.046	0.037
T_{max} (h)	6	6	11.0	11.0	317.3	318.7
$t_{½}$ (h)	28.5	23.2	38.5	37.4	38.8	27.9
$AUC_{0-\infty}$ (µg eq·h/ml)	1.01	0.78	81.7	61.1	11.0	8.45
Plasma						
C_{max} (µg eq/ml)	0.030	0.022	1.20	0.64	0.04	0.03
T_{max} (h)	6.7	5.3	15.0	10.7	317.3	318.5
$t_{½}$ (h)	27.3	19.5	36.3	35.3	34.8	23.9
$AUC_{0-\infty}$ (µg eq·h/ml)	0.87	0.61	69.8	44.6	0.83	0.57

From Powles & Thornley (1995)
AUC, area under the curve; C_{max}, maximum concentration; $t_{½}$, half-life; T_{max}, time to reach C_{max}

spleen, liver, lung and adrenal glands (1.1–5.9 µg eq/g tissue) 3 hours after a single low dose. By 24 hours after dosing, the highest concentrations in tissues were found in the pituitary gland (males 10.0 µg eq/g tissue, females 4.3 µg eq/g tissue) and adrenal glands, sublingual glands, Harderian glands, large intestine and lung (0.4–1.9 µg eq/g tissue). By 168 hours after dosing, almost all radioactivity concentrations had decreased to levels below 0.1 µg eq/g. After a single dose of 20 mg/kg bw, the distribution to the tissues followed a pattern similar to that observed following administration of 0.5 mg/kg bw, but with greater than 40 times higher concentrations. Administration of repeated low doses gave a similar tissue distribution as was observed for a single dose, with approximately 2 times higher concentrations than observed following a single low dose. Except for Harderian glands and pituitary gland, all concentrations had decreased below 1 µg eq/g by 168 hours after the final dose. In all groups, the lowest concentrations were measured in the brain and spinal cord.

The average recoveries of emamectin in tissues and excreta are shown in Table 4 (single dose) and Table 5 (repeated dose). During the first 12 hours after a single 20 mg/kg bw dose, high percentages of the administered dose were found in tissues (96–102% at 3 hours, 68–75% at 6 hours). The highest concentrations were found in the small intestine plus contents (51–61%) and in the carcass (14–18%) at 3 hours and in the carcass (26–27%) and caecum plus contents (11–15%) at 6 hours. Almost all MAB1a administered was excreted via faeces, starting at 24 hours after treatment. Excretion via faeces proceeded more quickly following a single low dose (87% after 48 hours) than following a single high dose (78% after 96 hours). After repeated dosing, faecal excretion was also the main excretion route, with maximal excretion at treatment day 7 (6–8% of the administered dose over the period of 144–168 hours). In animals killed 1 hour after the administration of the last dose, most emamectin was already excreted (~80%). In all treatment groups, low amounts of MAB1a were excreted in the urine and bile. Biliary excretion was measured for up to 48 hours after a single dose, but was not complete at this time. Following single or repeated administration, excretion routes were quantitatively similar in male and female rats (Powles & Thornley, 1995).

Groups of male and female Charles River Sprague-Dawley rats were treated with single or repeated oral gavage doses or intravenous administration of [3,7,11,13,23-^{14}C]MAB1a (683,825-003E003, purity ~94%), [5-^{3}H]MAB1a (L-683,825-001A006, purity ~97%) or unlabelled MK-0244 (L-656,748-038W002, purity 91.1% MAB1a and 5.1% MAB1b). The substances were dissolved in a propylene glycol/saline solution (1:1, v/v). The positions of the ^{3}H and ^{14}C labels are shown in

Table 4. Distribution of administered emamectin after a single dose (average males and females)

Time after dosing (h)	% of administered dose							
	Single low dose				Single high dose			
	Faeces	Urine	Bile	Tissues[a]	Faeces	Urine	Bile	Tissues[a]
1–12	1.6	0.01	0.7	68–102	—	—	0.6	78–96
24	65.7	0.06	0.7	38.4	32.6	0.06	0.7	—
48	19.9	0.04	1.0	—	17.6	0.06	1.1	—
60	—	—	—	—	—	—	—	31.8
72	7.6	0.03	—	—	16.8	0.04	—	—
96	2.9	0.02	—	—	10.7	0.02	—	—
120	2.8	0.01	—	—	5.8	0.01	—	—
144	1.1	0.00	—	—	3.8	0.02	—	—
168	0.7	0.01	—	1.4	2.0	0.01	—	3.6
Total	102.3	0.2	2.4	N/A	89.3	0.20	2.4	N/A

From Powles & Thornley (1995)
N/A, not applicable
[a] Wide variety of tissues, including contents of gastrointestinal tract.

Table 5. Distribution of administered dose of emamectin after 14 daily low doses (average males and females)

Time after first dose (h)[a]	% of administered dose		
	Repeated low dose		
	Faeces	Urine	Tissues
0–144	32.3	0.0	—
168–312	40.8	0.1	—
313	—	—	15.7
318	—	—	17.0
336	7.6	0.0	—
352	—	—	5.3
360	4.2	0.0	—
384	2.1	0.0	—
408	1.1	0.0	—
432	0.6	0.0	—
456	0.4	0.0	—
480	0.3	0.0	1.0
Total	89.4	0.1	N/A

From Powles & Thornley (1995)
N/A, not applicable
[a] Repeated dosing during 14 days (0–312 hours), once per day.

Figure 1. An overview of the study groups is presented in Table 6. Additionally, each group contained untreated control rats (one of each sex per group). Tissues (bone, brain, fat, gastrointestinal tract, heart, kidney, liver, lung, muscle, spinal cord, spleen, testes or uterus and ovaries), together with the tail, feet, skin and residual carcass of each rat, were collected. Radioactivity levels were determined by LSC. Metabolite characterization was performed in faeces, liver, kidney, muscle and fat isolated from group 1 and is described in section 1.2.

Table 6. Overview of experimental groups

Test group	Number and sex	Administration route	Dose	Sampling regime
1	6M/6F	Oral gavage	20 mg/kg bw (single), [^{3}H/^{14}C]MAB1a	Urine and faeces collected at 8 h and 1, 2, 3, 4, 5, 6 and 7 days after dosing; sacrifice and tissue collection on day 7
2	6M/6F	Oral gavage	0.5 mg/kg bw per day MK-0244 (repeated, 14 days) followed by a single dose of 0.5 mg/kg bw [^{14}C]MAB1a	
3	12M/12F	Oral gavage	0.5 mg/kg bw (single), [^{14}C]MAB1a	Blood samples at 2, 4, 8, 12 and 18 h (6M/6F) and at days 1, 2, 3, 4, 5, 6 and 7 (6M/6F) after dosing; urine and faeces collected at 8 h and 1, 2, 3, 4, 5, 6 and 7 days after dosing; sacrifice and tissue collection on day 1 (6M/6F) and day 7 (6M/6F)
4	12M/12F	Intravenous into femoral vein	0.5 mg/kg bw(single), [^{14}C]MAB1a	

From Mushtaq (1993)
F, female; M, male

MAB1a benzoate dosing solutions were homogeneous, and radiolabelled solutions remained stable during the study. Three deaths occurred among the rats dosed intravenously, owing to excessive bleeding or overdose of anaesthetic during blood collection: one male (day 4) and two females (day 1). No abnormalities were observed in the rats upon oral exposure, with the exception of one rat (high dose) that was sluggish for 4 hours after administration.

The kinetics results are summarized in Table 7. Maximum absorption by orally treated rats was reached at 12 hours (males) and 4 hours (females) after treatment, and levels had returned to background at 96 hours. Females showed longer plasma elimination half-lives compared with males. Comparison of AUC values following oral and intravenous dosing indicated that the oral bioavailability of MAB1a benzoate was 55% and 74% in male and female rats, respectively.

Nearly all MAB1a, whether orally or intravenously administered, was excreted in the faeces (95–106%). Excretion via urine was in total less than 1%, and residues measured in tissues were 4–6% at day 1 and 0.1–1.5% at day 7. No differences were found between males and females. MAB1a concentrations in faeces and urine peaked at 8–24 hours after treatment, with up to more than 1000-fold higher concentrations in faeces compared with urine. More than 90% of MAB1a was eliminated in the high-dose group within 5 days and in the low-dose groups within 2–3 days. No differences in elimination route and rate were found between the two administration routes. Radioactivity measured in tissues from day 1 and day 7 showed that MAB1a residue levels had decreased approximately 100-fold from day 1 until day 7, indicating a high elimination rate. The tissues with the highest residue levels were spleen, lung, gastrointestinal tract, kidney and liver. The lowest levels were observed in the brain and spinal cord. The distribution of MAB1a residues over the tissues was comparable among the four experimental groups, between the sexes and between the two administration routes at day 7. Intravenous administration resulted in higher residue levels compared with oral gavage. Furthermore, high dosing resulted in approximately 100 times higher residue contents in tissues compared with low dosing. The tissue residues were overall about 2 times higher in males than in females upon single or repeated low dosing, which was not the case in the high-dose group (Mushtaq, 1993).

In a study performed in accordance with OECD Test Guideline 417, only disposition of MAB1a was examined. Male and female Crl:CD(SD)BR rats were treated intravenously with a single [23-^{14}C]-MAB1a dose of 0.5 mg/kg bw (purity > 97.0%) diluted with emamectin (batch reference AMS 921/4, purity 94.4% MAB1a and 3.7% MAB1b). The position of the single ^{14}C label is indicated by the asterisk in Figure 1. The dose vehicle was propylene glycol/physiological saline (50:50, v/v). An overview of the experimental groups is presented in Table 8. Three days before dosing, rats in group 1 were

Table 7. Toxicokinetic data in plasma after a single low oral or intravenous dose administration of ^{3}H-labelled MAB1a

	Group 3: oral administration, single dose (0.5 mg/kg bw)		Group 4: intravenous administration, single dose (0.5 mg/kg bw)	
	Males	Females	Males	Females
C_{max} (ng eq/ml)	16.96	21.38	41.82	34.62
T_{max} (h)	12	4	2	2
$t_{½}$ (h)	34.36	51.05	28.64	40.66
$AUC_{0-\infty}$ (µg eq·h/ml)	893.46	1127.46	1635.32	1516.81
Bioavailability (%)[a]	54.6	74.3	—	—

From Mushtaq (1993)
AUC, area under the curve; C_{max}, maximum concentration; $t_{½}$, half-life; T_{max}, time to reach C_{max}
[a] Bioavailability (%) = 100 × (AUC (oral) / AUC (intravenous)).

Table 8. Experimental groups

Test group	Experiment	Number and sex	Administration route	Dose	Sampling regime
1	Bile duct cannulation	3M/4F	Intravenous	0.5 mg/kg bw (single)	Urine, faeces and bile were collected at 6, 24, 48 and 72 h after dosing; faeces were collected at 24, 48 and 72 h; termination at 72 h
2	Autoradiography	6M/6F	Intravenous	0.5 mg/kg bw (single)	Termination and tissue and blood collection at 2, 24 and 72 h

From Silcock (2005)
F, female; M, male

cannulated by surgically implanting cannulae into the bile duct and duodenum for collection of bile. Terminal blood samples were taken by cardiac puncture, and the gastrointestinal tract was removed. In group 2, the blood was collected from the tail vein prior to termination, and the gastrointestinal tract was stored for analysis. The tissue levels were analysed by autoradiography and LSC.

The mean administered dose was 0.52 mg/kg bw in the bile duct cannulation study and 0.55 mg/kg bw in the autoradiography study. Blood was observed in the urine of some rats on the day of dosing. Four rats in group 1 (one male, three females) were terminated at earlier time points (18, 48, 52 and 52 hours after dosing). No samples or data were available from six rats (three of each sex) in group 2.

In the bile duct–cannulated rats (test group 1), total recovery was 95–97% at 72 hours, of which 37–44% was found in the faeces, 34–37% in the carcass and 7–9% in the total gastrointestinal tract plus contents. In rats terminated earlier (18–52 hours), the highest percentages were found in carcass (58–69%) and gastrointestinal tract plus contents (11–15%). In the only female examined at 72 hours, a higher percentage of residue was found in faeces compared with the two males examined at 72 hours (45% female, 37–38% males). Biliary excretion was more extensive in males than in the female at 72 hours (5% female, 11–17% males). Urinary excretion was limited (0.6–1.5%). Excretion was not complete at 72 hours. At 2, 24 and 72 hours, MAB1a levels in blood were lower (~2–10 times) than levels in the gastrointestinal tract and gastrointestinal tract contents. Within the gastrointestinal tract, the highest concentrations of radioactivity were found in the contents. At 2 hours, the highest residue concentrations were found in the small intestine (duodenum, jejunum, ileum). Concentrations decreased in these parts of the gastrointestinal tract with time, but increased in the large intestine (caecum, colon and rectum), where the highest concentrations were found at 72 hours. The concentrations at 72 hours were low, suggesting that most of the substance had been excreted. No significant sex differences were found.

In test group 2, following intravenous administration of [^{14}C]MAB1a to non-cannulated rats, autoradiography of the excised gastrointestinal tract demonstrated that radioactivity was excreted mainly into the contents of the ileum and that the radioactive material progressed down the gastrointestinal tract over the time course of the experiment. The presence of radioactivity in the gastrointestinal tract and contents in the bile duct–cannulated rats and the results from the autoradiography experiment indicate that intestinal secretion is the main route of elimination for subsequent excretion in faeces. This is consistent with the known role (Laffont et al., 2002) of p-glycoprotein as an efflux transporter of avermectins (Silcock, 2005).

Dogs

Male Beagle dogs were dosed orally by gavage with emamectin benzoate salt (L-656,748-038W002, purity 96.9%) or emamectin benzoate monohydrate (L-656,748-052S011, purity 98.0%) suspended in 0.5% methylcellulose at a dose of 0.5 mg/kg bw. A crossover study was performed with two dogs per group. Group 1 received the benzoate salt at day 1 and the benzoate hydrate at day 15; group 2 received the benzoate hydrate at day 1 and the benzoate salt at day 15. Blood samples were taken at day 1 and day 15 and at 0.5, 1, 2, 4, 6, 8, 24, 48, 96 and 168 hours post-dosing. The plasma was used for kinetics analysis by high-performance liquid chromatography (HPLC).

No mortality or changes in body weight were observed in this study. The mean C_{max} was 33.0 ng/ml for benzoate salt and 35.2 ng/ml for the benzoate monohydrate, and these peak levels were reached after 2 and 4 hours, respectively. The AUC from 0 to 168 hours was on average 1502.6 and 1546.0 ng·h/ml for the benzoate salt and the hydrate, respectively (Gerson, 1992a).

In a second study in dogs, four male Beagle dogs were orally (gavage) administered emamectin benzoate salt (L-656,748-051P001, purity 98.8%) or emamectin hydrochloride (L-656,748-050M001, purity 98.7%) dissolved in ethanol/deionized water (final ethanol concentration 5%) and deionized water, respectively, at a dose of 0.5 mg/kg bw. After ^{3}H radiolabelling, the purity was 96.2–97.7% for both the benzoate salt and the hydrochloride. The tritium label is incorporated at the C5 position of MAB1a, as indicated by the asterisk in Figure 1. The same crossover principle was used as in the study described above with the same sample collection. The crossover study was performed with two dogs per group. Group 1 received the benzoate salt at day 1 and the hydrochloride at day 15; group 2 received the hydrochloride at day 1 and the benzoate salt at day 15. Blood samples were taken at day 1 and day 15 and at 0.5, 1, 2, 4, 6, 8, 24, 48, 96 and 168 hours post-dosing. Additionally, urine and faeces were collected at 0–24 and 72–96 hours post-administration.

No mortality or changes in body weight were observed. The mean C_{max} was 101 ng eq/g for the benzoate salt and 109 ng eq/g for the benzoate monohydrate. Peak levels were reached in 4–6 hours. The benzoate salt and hydrochloride had similar half-lives (35.7 and 35.5 hours, respectively) and similar AUCs (4479 and 4574 ng/g plasma per 7 days, respectively). Excretion of the two substances was similar. Approximately 40% of the total administered dose was excreted via faeces (~34% within the first 24 hours), and 0.01% via urine (Manson, 1992a).

1.2 Biotransformation

Rats

In the study of Powles & Thornley (1995) (see study description in section 1.1), metabolites were analysed in urine, faeces, bile, plasma and tissues. Metabolites were isolated by HPLC and identified by co-chromatography with authentic reference standards.

After oral administration of MAB1a, the main metabolite was AB1a, the des-*N*-methyl derivative of emamectin B1a. In all excretion samples and tissues measured, the majority of the radiolabel

co-chromatographed with MAB1a and only small proportions with AB1a, together accounting for approximately 90–99% of the radioactivity measured per sample. Very small proportions of polar material were found in faeces, urine and bile. Of all excreta, MAB1a and AB1a were found primarily in faeces, with MAB1a predominating (66–94%) and with a smaller proportion of AB1a (2–22%). MAB1a and the metabolite were also detected in plasma (78–100% MAB1a, 5–18% AB1a) and bile (49–68% MAB1a, 9–14% AB1a). In urine, MAB1a and metabolites were present at very low levels (0.001–0.011% of the administered dose). Both MAB1a and the metabolite AB1a were measured in most tissues observed, including abdominal fat, heart, Harderian glands, kidney, liver, lung, pancreas, skeletal muscle and spleen. MAB1a accounted for the highest levels (~65–90% of the radioactivity measured), and a smaller percentage was identified as AB1a (~10–30%). Levels of metabolites were comparable in males and females and between dose groups. Radioactivity that was not represented by MAB1a or AB1a was associated with polar material. It is concluded that biotransformation of MAB1a results in formation of the metabolite AB1a, which is present in lower amounts compared with the parent compound in various tissues and is excreted mainly via faeces.

In the in vivo study of Mushtaq (1993) (see study description in section 1.1), metabolites were analysed in faeces, liver, kidney, muscle and fat. Metabolites were isolated by reversed-phase HPLC and identified by chromatography with standard metabolite reference mixture, ultraviolet markers, mass spectrometry and nuclear magnetic resonance.

HPLC analyses revealed that the major portions of radiolabel in all the samples were associated with MAB1. The one identified metabolite found using HPLC analyses was AB1a. The relative proportions of MAB1a (50–95%) and AB1a (1–20%) were comparable between low- and high-dose administration and between single and repeated dosing. A time-dependent increase of the metabolite (measured as per cent radioactivity in HPLC fraction) was observed in faeces from day 1 (1–6% AB1a) until day 7 (11–21%). Males and females responded similarly, with the exception of lower percentages of AB1a following a single low dose in females compared with males. AB1a concentrations in liver, kidney, muscle and fat were higher than concentrations in faeces at day 1 (5–15%), but comparable at day 7 (4–23%). Males and females showed similar percentages of metabolite in their tissues, except for fat (~16% males, ~5% females). Furthermore, the percentage of AB1a in tissues was lower following intravenous administration compared with oral administration. MAB1a and AB1a together accounted for 91–98% of the radioactivity in the HPLC fractions. However, at day 7, only 60–70% of the radioactivity (repeated dose and single low dose group) could be explained by MAB1a and AB1a, owing to the detection of an unknown peak or multiple peaks. This was due to the low residue contents (Mushtaq, 1993).

The proposed metabolic pathway of emamectin benzoate is depicted in Figure 2.

2. Toxicological studies

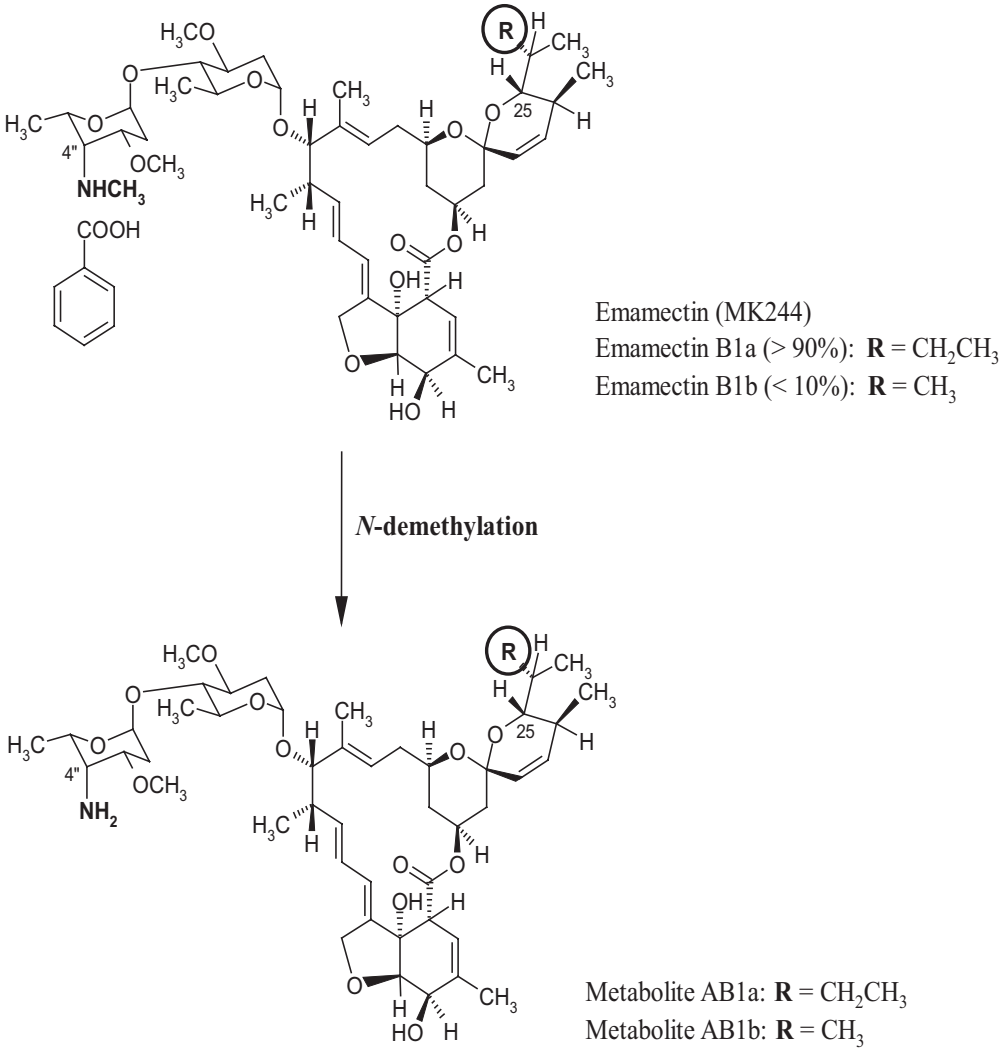
2.1 Acute toxicity

The results of studies of acute toxicity with emamectin benzoate are summarized in Table 9. Treatment-related mortality and signs of toxicity were observed during the acute oral, dermal and inhalation studies.

(a) Oral administration

In oral studies in mice and rats, clinical signs of toxicity observed in surviving animals were ptosis (mice only), hypoactivity, tremors, ataxia, salivation, irritability, bradypnoea, diarrhoea,

Figure 2. Proposed metabolic pathway of emamectin benzoate in the rat



AB1a/b = des-*N*-methyl derivative of emamectin B1a/b

anogenital staining, reduced faecal volume and weight loss. In mice and rats, clinical signs were observed at oral doses equal to 26 and 44 mg/kg bw, respectively, and higher.

(b) Dermal application

In dermal studies, clinical signs were induced by all dermal doses tested (500–2000 mg/kg bw). Toxicity symptoms similar to those seen in the oral studies were observed in the dermal studies, with additional signs of tonic convulsion, piloerection, decreased activity, hunched back, coloured discharge from nose and eyes and incoordination.

(c) Exposure by inhalation

In the acute inhalation study, signs of mild/moderate toxicity were observed during and after exposure, with comparable symptoms as described for the other studies.

(d) Dermal irritation

An acute dermal irritation study was performed according to OECD Test Guideline 404. Three New Zealand White rabbits (one male, two females) were dermally exposed for 4 hours to 0.5 g emamectin (purity 96.2%, batch ID SNA6B019, white powder) in a 75% weight per weight (w/w)

Table 9. Results of studies of acute toxicity with emamectin

Species	Strain	Sex	Route	Vehicle	Purity (%)	LD_{50} (mg/kg bw) / LC_{50} (mg/l)	Reference
Mouse	Crl:CD1 [ICR] BR	F	Oral	Aqueous methylcellulose	95.9	107	Galloway (1993b)[a]
Rat	Crl:CD(SD)BR	F	Oral	Carboxymethylcellulose	96.2	53	Bagdon (1993)[b]
Rat	Crl:CD(SD)BR	F	Oral	Carboxymethylcellulose	96.2	58	Bagdon (1993)[c]
Rat	Sprague-Dawley	F	Oral	Carboxymethylcellulose	96.2	237	Durando (2006a)[d]
Rat	Crl:CD(SD)BR	M/F	Oral	Deionized water	96.9	88 (M) 76 (F)	Lankas (1994a)[e]
Rat	Crl:CD(SD)BR	M/F	Oral	Sterile water	96.9	67 (M) 70 (F)	Manson (1992d)[f]
Rat	Sprague-Dawley	M/F	Dermal	Distilled water	96.2	> 2000	Durando (2006b)[g]
Rat	Wistar	M/F	Dermal	None	96.6	500–1000 (M) 1893 (F)	Zelenak (2010)[h]
Rat	HsdRccHanTM: WIST (Wistar-derived)	M/F	Inhalation	None	96.2	> 1.049 (M) 0.663 (F)	Noakes (2006)[i]

F, female; LC_{50}, median lethal concentration; LD_{50}, median lethal dose; M, male

[a] Reported as a range-finding experiment in an in vivo micronucleus study. Study performed with emamectin benzoate, batch L-656,748-052S002. Clinical signs were observed at 26 mg/kg bw and higher. No clinical signs were observed at 8 mg/kg bw.

[b] Performed with emamectin benzoate salt (L-656,748-038W lot No. 2, purity 96.4%) at 40, 60, 90 and 135 mg/kg bw. Mortality was seen after 60, 90 and 135 mg/kg bw. Clinical signs were observed in all treatment groups and included ataxia, tremors, ptosis, bradypnoea, decreased activity (except at 40 mg/kg bw), salivation, blood-like staining, urine staining, loss of righting reflex and lateral recumbency, and soft stools (only at 135 mg/kg bw).

[c] Performed with emamectin benzoate hydrate (L-656,748-052S, purity 99.1%) at 40, 60, 90 and 135 mg/kg bw. Mortality was seen after 60, 90 and 135 mg/kg bw. Clinical signs were observed in all treatment groups and included ataxia, tremors, ptosis, bradypnoea (except at 40 mg/kg bw), decreased activity, salivation, blood-like staining, urine staining, loss of righting reflex and lateral recumbency, and soft stools (except at 60 mg/kg bw).

[d] Performed according to OECD Test Guideline 425 with emamectin benzoate, batch ID SNA6B019. At 23.7 and 75 mg/kg bw, no rats died, lost body weight or showed clinical signs of intoxication. Rats dosed at 237 mg/kg bw (n = 3, 2 died, 1 survived) and 750 mg/kg bw (n = 1, died) showed hypoactivity, tremors, diarrhoea, anogenital staining and reduced faecal volume.

[e] Performed according to OECD Test Guideline 401 with emamectin hydrochloride (L-656,748-010V003). Clinical signs were observed at doses of 44.4 mg/kg bw and higher. No pathological examination was performed.

[f] Performed as an acute neurotoxicity study. Rats (10 males and 10 females per group) were treated by gavage with a single dose of emamectin hydrochloride salt (L-656,748-010V003, purity 96.9%) at 0, 27.4, 54.8 or 82.2 mg/kg bw. For a detailed description, see section 2.6.

[g] Performed according to OECD Test Guideline 402 with emamectin benzoate, batch ID SNA6B019. Five males and five females received a dermal application of 2000 mg/kg bw. An additional group of five females received 1000 mg/kg bw. At 2000 mg/kg bw, two of five females died. All males at 2000 mg/kg bw and all females at 1000 mg/kg bw survived. Surviving animals in the 2000 mg/kg bw group showed clinical signs, including hypoactivity, tremors and/or ataxia, with recovery by day 12. All females dosed with 1000 mg/kg bw showed irregular respiration, hypoactivity, tremors and/or reduced faecal volume, with recovery by day 11.

[h] Performed according to OECD Test Guideline 402 with emamectin benzoate, batch ID SNA6A015. Groups of five males and five females received emamectin by dermal application at 500, 1000 or 2000 mg/kg bw. Mortalities in the males of the low-, mid- and high-dose groups were 1/5, 3/5 and 1/5, respectively. Mortalities in the females of the low-, mid- and high-dose groups were 0/5, 0/5 and 3/5, respectively. Clinical signs of toxicity were seen in animals dosed at 500, 1000 and 2000 mg/kg bw from 2 days after the treatment and included vocalization, irritability, tremors, tonic convulsion, piloerection, decreased activity, hunched back, coloured discharge from nose and area around eyes and incoordination. Additionally, prone position, dyspnoea and laying on the side were noted in some animals dosed at 1000 and 2000 mg/kg bw. Some clinical signs still persisted in most animals at the end of the observation period.

[i] Performed according to OECD Test Guideline 403 with emamectin benzoate, SNA6B019.

mixture with distilled water. Test mixture (0.67 g) was placed on a 2.5 cm × 2.5 cm, 4-ply gauze pad and applied to one 6 cm^2 intact dose site on each animal. The pad and entire trunk of each animal were then wrapped with semi-occlusive 7.6 cm Micropore tape to avoid dislocation of the pad. After 4 hours of exposure of the rabbits to the test substance, the pads and collars were removed, and the

test sites were gently cleansed of any residual test substance. Dermal irritation was scored according to the Draize system at 30–60 minutes and 24, 48 and 72 hours after patch removal.

Slight erythema was observed for all three rabbits within 1 hour after patch removal. All rabbits were free of irritation at 72 hours post–patch removal (Durando, 2006c). The Meeting concluded that emamectin is slightly irritating to the skin.

(e) Ocular irritation

In an acute eye irritation study, performed according to OECD Test Guideline 405, 0.1 ml emamectin (96.2% purity, batch ID SNA6B019) was instilled into the conjunctival sac of the right eye of three New Zealand White rabbits (two males, one female). The untreated left eye served as a control. The eyes were not rinsed after instillation of emamectin. Eye irritation was scored according to the Draize system at 30–60 minutes, 24, 48 and 72 hours, and 4 and 7 days post-instillation.

One hour after the instillation of emamectin, iritis and conjunctivitis were observed in all treated eyes. At 24 hours, corneal opacity was observed. At day 4, two rabbits still had symptoms. All rabbits were free of irritation at 7 days post-instillation (Durando, 2006d). The Meeting concluded that emamectin is moderately irritating to the eye.

(f) Dermal sensitization

A dermal sensitization study (local lymph node assay) was performed according to OECD Test Guideline 429. CBA/Ca/Ola/Hsd female mice (four per group) were treated for 3 consecutive days with emamectin (purity 96.2%, batch ID SNA6B019) dissolved in dimethyl formamide at concentrations of 0.5%, 1% or 2.5% weight per volume (w/v) emamectin applied in a volume of approximately 25 µl to the dorsal surface of each ear. The draining auricular lymph nodes were examined for T lymphocyte proliferation.

The isotope incorporation was less than 3-fold at all dose levels compared with the vehicle control. There was no effect on body weight. Treatment with the positive control, hexylcinnamaldehyde, resulted in greater than 3-fold isotope incorporation (Betts, 2006). The Meeting concluded that emamectin is not a skin sensitizer.

2.2 Short-term studies of toxicity

Mice

In a 13-week dietary range-finding study, Crl:CD-1 BR mice (15 of each sex per group) received emamectin benzoate (L-656,748-038W002, purity 91.1% MAB1a and 5.1% MAB1b) in the diet. Emamectin concentrations were adjusted during the study to achieve the doses 0, 0.5, 1.5, 4.5 and 15 mg/kg bw per day. At the beginning of week 8, the dose level for the 1.5 mg/kg bw per day group was increased to 10 mg/kg bw per day in order to better define the toxicity. The actual average emamectin doses were 0, 0.5, 1.5, 4.6, 10.4 and 15.4 mg/kg bw per day.

Mice were observed daily for mortality and clinical signs. Body weight was measured before the first treatment and once per week during the study. Feed consumption was measured weekly. Ophthalmological examinations were conducted on all mice before the start of the study and on all control and high-dose mice in week 12. Blood was collected from all remaining mice at necropsy for haematological examinations and serum biochemical determinations. All mice underwent complete necropsy. Histopathology was performed on tissues from five control and five high-dose mice. No statistical analyses were performed.

One female (15 mg/kg bw per day, week 5) and one male (15 mg/kg bw per day, week 10) died during the study without any clinical signs. A cause of death could not be determined as a result of autolysis. No clinical signs were observed in any of the mice. The average body weight gain in the

15 mg/kg bw per day group at the end of the study was 41% lower in males and 21% lower in females compared with control mice. No effects on body weight gain were observed in the other treatment groups. During week 2, decreases in average body weight and feed consumption were observed in most groups (including controls), which were attributed to a decrease in feed consumption because of a technical problem with the feeders. During the other weeks, feed consumption was similar in all experimental groups. Changes were observed in haematological parameters in the highest-dose group compared with the control group; however, these changes were small and not consistent between males and females. Therefore, they are considered to be not toxicologically relevant. Glucose levels decreased by 10–17% at 1.5/10 mg/kg bw per day (females), 4.5 mg/kg bw per day (males) and 15 mg/kg bw per day (females + males) compared with the control group. However, the variation between mice within the groups was high, and the decreases were considered not toxicologically relevant. Creatinine, potassium, chloride, cholesterol, bilirubin, calcium, phosphorus, triglyceride and alkaline phosphatase levels were not determined. No ophthalmological effects were observed. Relative liver weight was increased (16%) in the male high-dose group, which may be related to the decrease in body weight gain. In the histopathology performed on tissues from mice in the control and high-dose groups, no treatment-related histological changes were observed (Gerson, 1992b).

Rats

Sprague-Dawley rats (10 of each sex per group) were fed diets containing emamectin hydrochloride salt (L-656,748-010V003, purity 96.9%) for 3 weeks at a concentration of 0, 5, 25, 50 or 100 ppm (equal to average doses, sexes combined, of 0, 0.5, 2.4, 4.9 and 10.5 mg/kg bw per day). The highest dose (100 ppm) was increased to 200 ppm (equal to an average dose, sexes combined, of 16.5 mg/kg bw per day) in week 2, because no treatment-related clinical signs of toxicity were observed. Rats were examined daily and were weighed before the test and weekly during the study. Feed consumption was measured in weeks 1 and 2 for a 5- to 6-day period. Haematology, serum biochemical determinations and urinalyses were performed on day 14 or 15. Blood was collected by orbital bleeding.

Three rats (two males, one female) died during the study. One high-dose male rat might have died because of treatment, because it showed loss of body weight (37 g) and feed intake after the substance concentration was increased to 200 ppm. One control female probably died as a result of the anaesthesia during blood sampling. One male that injured its mouth on the feeder was killed for humane reasons. A few days after increasing the highest dose level to 200 ppm, treatment-related physical signs, including tremors, decreased activity and salivation, were observed. Males and females dosed with 100 ppm emamectin had a lower weight gain compared with controls (week 1) and showed weight loss in week 2 after dosing with 200 ppm. Feed consumption was lower in males and females of the highest-dose group. Body weight gain and feed consumption in the other groups were comparable.

Haematological examination showed a significant decrease (48–63%) in concentrations of leukocytes in males and females in the highest-dose group compared with the control group ($P \leq 0.05$). Leukocyte concentrations in females were also decreased (23%) in the 50 ppm emamectin group. This could be mainly attributed to a decrease in lymphocytes in these groups. Furthermore, neutrophil levels were decreased (21–38%) in female rats treated with 50 or 100/200 ppm ($P \leq 0.05$). The concentrations of erythrocytes and haemoglobin and the per cent haematocrit were increased (9–18%) in males and females given the highest dose. Serum biochemistry analyses showed several significant ($P < 0.05$) changes in values measured in the 100/200 ppm group. Concentrations of glucose, urea nitrogen, total protein, aspartate aminotransferase (AST) (males only), alanine aminotransferase (ALT) and cholesterol were increased in males and females, which might indicate liver damage. Furthermore, alkaline phosphatase, chloride and potassium concentrations were decreased. No treatment-related effects were measured via urinalyses. Histopathological examinations were not performed (Lankas, 1992a).

In a 90-day dietary study performed in accordance with OECD Test Guideline 408, groups of 20 male and 20 female Sprague-Dawley rats were administered emamectin hydrochloride (L-656,748-010V003, purity 92.8% MAB1a and 4.1% MAB1b) orally for 90 days in the diet at variable concentrations, aimed to obtain the desired doses of 0, 0.5, 2.5 and 12.5 mg/kg bw per day. The high dose level was lowered to obtain a dose of 8 mg/kg bw per day in week 3 and further lowered to obtain a dose of 5.0 mg/kg bw per day in week 9, owing to decreases in feed consumption and weight gain. The final dietary doses were equal to 0, 0.5, 2.5 and 4.8 mg/kg bw per day in males and 0, 0.5, 2.6 and 5.1 mg/kg bw per day in females. The rats were observed daily for mortality and clinical signs. Feed consumption was measured every week. Rats were weighed before the test and weekly during the study. Ophthalmological examinations were conducted on all control and high-dose rats in week 4 and week 10/11. Blood was collected under ether anaesthesia by orbital bleeding from 15 males and 15 females from each treatment group at weeks 5, 8 and 12 for haematological examinations and serum biochemical determinations. Urinalysis was performed in weeks 8 and 12 (10 rats of each sex per group). Complete necropsy was performed on all rats, and selected organs were weighed. Histological examination was performed on a wide range of tissues in control and high-dose rats and on target organs (bone, skeletal muscle, brain, spinal cord, optic and sciatic nerve) and gross lesions of all rats. No statistical analyses were performed.

Nine male rats from the high-dose group were killed in weeks 3–11 as a result of emaciation and poor appearance, associated with decreased feed consumption and decreased body weight gain. These effects are considered treatment related. The deaths of one control and one mid-dose male and one control, one low-dose and two high-dose females were not substance related. Clinical signs of neurotoxicity were observed in the high-dose group in both males and females. These included tremors (from week 1 onward, decrease in tremors following lowering of the dose), splaying and limited use of hindlimbs (weeks 7, 9 and 11–14) and urine staining (from week 2 onward). Other clinical signs that were recorded were not considered treatment related. Rats treated with the target emamectin dose of 12.5 mg/kg bw per day (actual doses were 11.0 and 11.6 mg/kg bw per day in males and females, respectively) showed a decrease in weight gain (31% males, 29% females) in week 2 compared with the controls. In week 8, treatment with the target dose of 8 mg/kg bw per day (actual doses were 6.8 and 7.3 mg/kg bw per day in males and females, respectively) also resulted in a decreased weight gain (39% males, 32% females). At the end of the study, the total weight gain of males and females in the high-dose group was 50% lower than the weight gain in control group rats. The average feed consumption in the high-dose group was decreased (17–20%) during the whole study compared with the controls. No effects on body weight or feed consumption were observed in the low- or mid-dose group. Decreased concentrations of polymorphonuclear leukocytes (7–26%, males + females), monocytes (9–40%, males + females) and lymphocytes (16–23%, males) were observed in the high-dose group compared with the control group. These decreases became more pronounced over the treatment period. In the male high-dose group (weeks 5 and 8), increased levels (2–8%) of erythrocytes, haemoglobin and per cent haematocrit were found. In high-dose males and females, serum biochemistry revealed decreased glucose concentrations (22–31%), increased urea nitrogen (4–26%) and slightly decreased creatinine levels, and urinalysis showed a 50–60% reduction in urine volume. Except for changes in leukocytes, these haematological, serological and urinary changes are indicative of dehydration and reduced feed intake. Most absolute organ weights were decreased in the high-dose group compared with controls, whereas organ weights relative to body weight were increased. Organ weights relative to brain weight were unchanged, indicating that the absolute increases were an effect of decreased body weight gain. Gross changes observed in the high-dose group were emaciation (9 males, 2 females) and decreased muscle mass (11 males, 15 females). Histological examination revealed treatment-related neurological lesions in the high-dose group, consisting of cytoplasmic vacuolation of neurons in the brain (15 males, 16 females) and spinal cord (20 males, 18 females), degeneration in the spinal cord (10 males, 5 females) and degeneration of the sciatic nerve (17 males, 18 females). In the mid-dose group, cytoplasmic vacuolation of neurons in the brain was

observed in two males. Very slight optic nerve degeneration was seen in a high-dose female and a mid-dose male rat. In addition, atrophy of the skeletal muscle (20 males, 20 females) and trabecular atrophy in bone (17 males, 9 females) were found solely in the high-dose group (Lankas, 1992b).

The no-observed-adverse-effect level (NOAEL) in the 90-day dietary study in rats with emamectin was 0.5 mg/kg bw per day, based on the finding of cytoplasmic vacuolation of neurons in the brain observed in males at 2.5 mg/kg bw per day.

In a 1-year oral toxicity study in rats performed according to OECD Test Guideline 452, Crl:CD(SD)BR rats (20 rats of each sex per group) were treated via diet with emamectin benzoate (L-656,748-052S002, purity 92.5% MAB1a and 5.3% MAB1b). Dietary concentrations were adjusted weekly to achieve the intended dose levels of 0, 0.1, 1.0 and 5.0 mg/kg bw per day, and the actual average doses were as intended. The high-dose females received 5.0 mg/kg bw per day from the start until week 18, at which time the dose was reduced to 2.5 mg/kg bw per day as a result of treatment-related toxicity (see below). All rats were observed daily for mortality and clinical signs. Rats were weighed pre-test and once per week thereafter. Feed consumption was measured weekly. Ophthalmoscopic examinations were performed pre-test on all rats and in week 50 in the control and high-dose groups. Haematological examinations, serum biochemistry and urinalyses were conducted in weeks 13, 26 and 52 on 10 rats of each sex per group. Neurotoxicological evaluations were conducted on 10 rats of each sex per group in weeks 14, 24, 38 and 51 and included a functional observational battery (FOB) and motor activity testing. All rats underwent complete necropsy at termination, and selected organs were weighed. Tissues from the control and high-dose groups were further examined by histopathology. Statistical testing was done on neurotoxicological data, final body weight and liver weight.

No treatment-related mortality was observed. Clinical observations showed tremors (9 females, weeks 9–21), unkempt coat (11 females, weeks 12–20) and unsteady gait (3 females, weeks 15–24) in high-dose females after dosing with 5.0 mg/kg bw per day. After lowering the dose to 2.5 mg/kg bw per day in week 18, these clinical signs gradually declined over a period of 2–6 weeks. No other treatment-related clinical signs or ophthalmological changes were observed. Body weight loss (9 g loss on average) in the female high-dose group (5.0 mg/kg bw per day) was observed in weeks 12–18. During this period, control females gained 9 g. Body weight of the high-dose females increased after the dose was lowered to 2.5 mg/kg bw per day. The terminal body weights in the mid- and high-dose females were 7–8% higher than in control females. Males in the mid- and high-dose groups showed an increased body weight gain until week 48, with an increase up to 10% in the mid-dose group and 8% in the high-dose group compared with the controls. No treatment-related effects on feed consumption were found. Small increases and decreases in levels of polymorphonuclear leukocytes, lymphocytes and eosinophils were not dose related and not consistent between males and females and are therefore considered to be not toxicologically relevant. Glucose levels were slightly increased in males (7–18%) and females (6–10%) in a dose-related manner at all three time points measured. Triglyceride levels were increased in the high-dose males in week 52 (> 2-fold higher) compared with control levels. A dose-related increase in urine volume in males was observed in weeks 26 and 52, with 16–53% higher volumes in the mid- and high-dose groups compared with the controls. Organ weights were not affected by treatment. Examination (in 20 males and 20 females) of gross lesions showed a slight increase in rats with limb/foot sore in the male high-dose group (1 in control group, 5 in high-dose group). The main histopathological findings were the incidence of neuron degeneration in the brain (9 males, 19 females) and in the spinal cord (4 males, 2 females) in the high-dose group, which were absent in the other groups. Furthermore, increases in liver cellular infiltration (7 males control, 14 males high dose) and vacuolation in the centrilobular region of the liver (2 males control, 8 males high dose) were observed. Grip strength of the forelimb was 30% decreased in the female high-dose group in week 14. Decreased arousal was observed in 9 of 10 high-dose males in week 51. The Meeting considered other small effects observed in the neurotoxicity tests not treatment related.

The NOAEL was 0.1 mg/kg bw per day, based on increases in body weight gain observed at 1.0 mg/kg bw per day (Gerson, 1992c).

Dogs

A 14-week oral toxicity study was performed with dogs (four of each sex per dose) performed in accordance with OECD Test Guideline 409. Beagle dogs were administered emamectin hydrochloride (L-656,748-010V003, purity 96.9%) by gavage at initial doses of 0, 0.5, 1.0 and 1.5 mg/kg bw per day, which were reduced to 0, 0.25, 0.5 and 1.0 mg/kg bw per day after 2 weeks because of the severity of the observed toxicity. Dogs were observed daily for mortality and clinical signs. Feed consumption was measured 4 times per week and body weight weekly during the study. The dogs were subjected to ophthalmoscopy (pre-test, weeks 4 and 12), haematology and blood chemistry (pre-test, weeks 4, 8 and 12) and urinalysis (pre-test, weeks 8 and 12). Electrocardiograms were recorded prior to the study and in weeks 4, 8 and 12, approximately 3–5 hours after dosing. All dogs underwent complete necropsy, and selected organs were weighed. Histopathology was performed on a wide range of tissues in all dogs in the control and high-dose groups.

Three dogs (one male, two females) from the high-dose group, showing tremors, mydriasis, anorexia, lethargy and recumbency from weeks 2–3 onwards, were killed prematurely for humane reasons. The toxic signs progressed in severity until sacrifice. Owing to the severity of the clinical findings, all doses were reduced in week 2 (middle and high doses) and week 3 (low dose). Overall, treatment-related clinical observations were found only in the high-dose group. The surviving five dogs of the high-dose group revealed tremors in weeks 2–6 (2/5) and weeks 9–14 (2/5). In most cases, ataxia occurred simultaneously with the tremors. In week 3, a loss in body weight (10%) was observed in the high-dose group. After the dose was reduced, the body weight increased, and during the rest of the study, a weight gain similar to that of the control group was observed. Dogs in the high-dose group had a decreased total body weight gain (32% lower compared with the controls) at the end of the study. Feed consumption was decreased in weeks 2 and 3 in the high-dose group, especially in the prematurely sacrificed dogs. Dose reduction resulted in an improvement of feed consumption towards a level similar to the consumption in the control group. Body weight gain and feed consumption in the low- and mid-dose groups were comparable to those of the control group. No treatment-related ocular lesions or changes in the electrocardiograms or haematological parameters were observed. Serum biochemistry and urinalysis did not show treatment-related changes. The increased relative liver, kidney and thymus weights in the high-dose group are considered to reflect the reduced body weight of the dogs. Multiple treatment-related histological changes were found in the mid- and high-dose groups. Degeneration of neurons, nerves or white matter was found in the brain (three males, three females), spinal cord (four males, four females), sciatic nerve (four males, three females) and eye (two males, three females) in the high-dose group, and degeneration of the white matter was observed in the brain (three males, one female) and spinal cord (one male) in the mid-dose group. In addition, atrophy of the skeletal muscles was found in the mid-dose group (one male, one female) and high-dose group (three males, four females). These effects were absent in the control and low-dose groups. Other microscopic lesions observed were atrophy of the thymus (high dose: one male, two females) and a decreased number of erythropoietic cells in bone marrow (high dose: one male, two females). These lesions were observed only in the dogs that were prematurely killed and are probably secondary to the overall poor condition of the dogs (Lankas, 1994b).

The NOAEL in the 14-week gavage study in dogs with emamectin hydrochloride was 0.25 mg/kg bw per day, based on the finding of degeneration of central and peripheral nerve tissue and skeletal muscle, thymic atrophy and reduced bone marrow erythropoiesis at 0.5 mg/kg bw per day.

A 53-week oral toxicity study was performed in dogs according to OECD Test Guideline 452. Beagle dogs (four of each sex per group) were dosed by gavage with emamectin benzoate (L-656,748

038W002, purity > 97%) at 0, 0.25, 0.5, 0.75 or 1.0 mg/kg bw per day. The group receiving 0.75 mg/kg bw per day was added 4 weeks after the start of the study. All dogs were clinically examined daily just before and after dosing and between 4 and 6 hours after dosing. Although it was reported that neurological examination (gait, postural reactions, cranial nerve function and spinal nerve reflexes) was performed at several time points during the study, the results of these examinations were not reported. Body weight was measured pre-test, once in week 1 and twice per week thereafter. Feed consumption was measured weekly. Ophthalmological, haematological, serum biochemical and urinary examinations were performed pre-test and 3–4 times during the study. All dogs were necropsied, and weights of brain, liver, adrenals, kidneys, testes and thyroid were recorded. Histology was performed on a large selection of organs from all dogs, except for dogs treated with 0.25 mg/kg bw per day (males and females) and 0.5 mg/kg bw per day (females), from which only target organs (skeletal muscle, brain, spinal cord, peripheral nerves) were examined.

All dogs from the 1.0 mg/kg bw per day group and all males from the 0.75 mg/kg bw per day group were killed at day 23 and at day 50, respectively, owing to clinical signs of severe toxicity, including fine whole-body tremors, mydriasis, stiffness of hind legs, decreased motor activity, decreased feed consumption and body weight loss. Clinical signs were first observed during week 2 of treatment in the high-dose group. No mortality was observed in the other groups. Clinical signs were also observed in the females of the 0.75 mg/kg bw per day group (tremors, mydriasis, stiffness in hind legs, difficulty getting up, ataxia and hyper-reaction to touch) and in the females of the 0.5 mg/kg bw per day group (tremors, stiffness in hind legs). Body weight loss was observed from week 3 onward in the 1.0 mg/kg bw per day group and in males dosed with 0.75 mg/kg bw per day. The other groups had comparable weight gains during the study. Feed consumption was decreased in females after administration of emamectin at 1.0 mg/kg bw per day and in males after dosing at 0.75 mg/kg bw per day. Extra feed (soft feed or regular feed) was given to some of the dogs from all study groups, based on decreased feed consumption. Body weight and feed consumption may therefore not be representative. Decreases were observed in the levels of leukocytes (29% decrease), polymorphonuclear leukocytes (33% decrease) and eosinophils (72% decrease) in week 52 in females of the 0.75 mg/kg bw per day group compared with controls. Serum biochemistry revealed a decrease in glucose (−32%) and nitrogen urea (−26%) in the 0.75 mg/kg bw per day females in week 52, which might indicate malnutrition. Slight increases in potassium, calcium and phosphorus levels were also found at 0.75 mg/kg bw per day in females (week 52), which might be indicative of kidney failure; however, no treatment-related changes were found by urinalysis. Ocular examination revealed no effects of treatment. No treatment-related macroscopic changes were observed. Relative and absolute liver weights were increased in females after administration of emamectin at a dose of 0.75 mg/kg bw per day, but there were no accompanying histological changes. Degenerative lesions were found in central and peripheral nerve tissue and muscle tissue in the groups treated with 0.5, 0.75 and 1.0 mg/kg bw per day. These observations included retinal degeneration of ganglionic cells and axonal degeneration of the optic nerve at 0.75 and 1.0 mg/kg bw per day (Table 10) (Gillet, 1992).

The NOAEL in the 53-week gavage study in dogs with emamectin was 0.25 mg/kg bw per day, based on clinical signs in females and the finding in both sexes of central, optic and peripheral nerve degeneration and muscle degeneration at 0.5 mg/kg bw per day.

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a carcinogenicity study performed according to OECD Test Guideline 451, male and female Crl:CD-1 BR mice (50 of each sex per group) were administered emamectin benzoate (L-656,748-052S002, purity 97.8%) via the diet for 79 weeks. Concentrations in the diet (0, 1.8–4.1, 9–20 and

Table 10. Histological findings in dogs after 53 weeks of dietary treatment with emamectin

	Dose (mg/kg bw per day)									
	0		0.25		0.5		0.75		1.0	
	M	F	M	F	M	F	M	F	M	F
Number necropsied	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*
Focal neuron degeneration, brain	—	—	—	—	—	—	2	—	1	2
Axonal degeneration, brain	—	—	—	—	1	2	4	3	4	4
Axonal degeneration, spinal cord	—	—	—	—	—	2	4	2	—	4
Axonal degeneration, sciatic nerve	—	—	—	—	3	1	4	4	4	4
Degeneration, retina	—	—	—	—	—	—	2	1	3	3
Axonal degeneration, optical nerve	—	—	—	—	—	—	3	1	4	3
Degeneration, muscle fibres	—	—	—	—	0	1	1	3	—	—

From Gillet (1992)
F, female; M, male

64–97 ppm for males; 0, 2.1–3.7, 11–18 and 55–74 ppm for females) were adjusted to obtain the desired doses of 0 (two identical control groups per sex), 0.5, 2.5 or 12.5 mg/kg bw per day. The high dose was reduced to 7.5 mg/kg bw per day (week 9 males, 45–49 ppm; week 48 females, 16–36 ppm) as a result of severe toxicity (clinical signs, weight loss) and was further reduced to 5.0 mg/kg bw per day in males in week 31 (13–31 ppm). At the nominal doses of 0.5, 2.5, 5.0, 7.5 and 12.5 mg/kg bw per day, the actual mean emamectin doses in males were 0.5, 2.6, 5.1, 7.5 and 12.8 mg/kg bw per day. At the nominal doses of 0.5, 2.5, 7.5 and 12.5 mg/kg bw per day, the actual mean emamectin doses in females were 0.5, 2.5, 7.6 and 12.6 mg/kg bw per day. Mice were examined daily for mortality and weekly for clinical signs. Body weight and feed consumption were measured weekly. An additional 30 mice (15 of each sex) per group were used for haematological determinations, performed in week 52, and were discarded without further examination. Ophthalmological examinations were performed before the test and in weeks 38–39 and 77. All mice were killed for complete necropsy in week 79, and brain, liver, kidney, adrenal glands and testes of 10 surviving mice were weighed. A detailed set of tissues of all mice was microscopically examined.

Mortality was significantly increased in the high-dose group ($P < 0.001$), with 68% mortality in males (34% in male control group) and 60% in females (25% in female control group). Six high-dose males died in weeks 3–11. Prior to death, these mice vocalized and had developed tremors. These clinical signs were not present in the other groups. The most frequently observed cause of death or reason for killing was an infectious process (dermatitis, abscess), which occurred from week 52 onwards, more commonly in males than in females of all groups, suggesting that the condition was exacerbated by the normal fighting among group-housed males and probably unrelated to treatment. The incidences of skin lesions and number of deaths related to infection were increased in high-dose males and females. The lesions showed heavy growth of *Staphylococcus intermedius* and/or *Streptococcus* group G, which were unresponsive to treatment with antibiotics. Next to tremors and vocalization, treatment-related clinical signs included a fine fasciculating tremor of the forequarters/forelimbs that was observed in the high-dose group (70–90%) and was absent in the other groups. The average weight gain was significantly lower in the high-dose males and females compared with the control mice ($P < 0.001$). In addition, weight loss was observed in high-dose males (weeks 23–60) and high-dose females (weeks 30–55). Feed intake in the high-dose groups was approximately 10% lower compared with control mice; however, a similar decrease was also observed in the low- and mid-dose groups, which had normal weight gains. This indicates that the decreased body weight gain observed in the high-dose groups was not caused by reduced feed consumption. Ophthalmological examination

revealed a high incidence of blepharitis only in the high-dose groups (11/50 males, 9/50 females). Haematology showed a small decrease (14–17%) in erythrocytes, haemoglobin and haematocrit in high-dose males. Neutrophil concentrations were increased (week 79, females) and lymphocyte and leukocyte concentrations were decreased (week 79, males) after high-dose treatment. These changes might have been related to the increased incidence of infections, which, although occurring in all dose groups, were more common at the high dose. Relative (to body weight) liver, kidney and adrenal weights were increased in the high-dose group, whereas absolute organ weights were similar or decreased compared with controls. These effects are probably related to the decreased body weight in the high-dose group. No treatment-related increase in the incidence of tumours was observed in any of the tissues examined. In the high-dose group, increased incidences of prolapsed penis (9/50 high dose, 1/50 controls), enlarged spleen (24 high dose, 4–9 controls), enlarged lymph node (23–27 high dose, 5–8 controls) and dermatitis (males: 35/50 high dose and 13/50 controls; females: 36/50 high dose and 2/50 controls) were observed during gross observations. Microscopic examination of animals found dead, killed preterm for humane reasons or killed at termination showed that the incidences of dermatitis and other infection-related findings, including peritonitis, liver abscess, heart abscess and keratitis, were increased in the high-dose group. A 2- to 3-fold higher incidence of extramedullary haematopoiesis in the spleen, hyperplasia of lymph nodes and myeloid hyperplasia in the bone marrow was found in both males and females in the high-dose group compared with controls. This increase is probably a reaction to the infections. In two high-dose males that died on days 55 and 60, lesions of the sciatic nerve, characterized by vacuolation and the presence of small myelin balls in the nerve fibres, were observed.

The Meeting considered all neoplastic findings, both malignant and benign, occurring at 79 weeks incidental to treatment with emamectin, as the incidences in treated and control groups are similar and the intergroup distribution shows no relationship to dose level.

The NOAEL in the 79-week dietary study in mice with emamectin was 9–20 ppm, equal to a dose level of 2.5 mg/kg bw per day, based on increased mortality, reduced body weight gain and weight loss, changes in haematology and increased incidence of infectious diseases (mainly dermatitis) and lesions in the haematopoietic/lymphoreticular system and sciatic nerve, observed at 5.1 mg/kg bw per day (Lankas, 1994c).

Rats

In a 105-week combined toxicity and carcinogenicity study performed according to OECD Test Guideline 453, Sprague-Dawley rats (controls 130 of each sex, treatment groups 75 of each sex per dose) were fed diet with emamectin benzoate (L-656,748-052S, lot No. 2, purity 95.9%). Dietary concentrations were adjusted during the study to achieve the targeted doses of 0, 0.25, 1.0 and 5.0 mg/kg bw per day. The high dose was reduced to 2.5 mg/kg bw per day in week 6 (males) and week 10 (females) because of unacceptable weight loss and tremors observed in a 14-week neurotoxicity study with rats (see section 2.6). The actual mean emamectin doses were 0, 0.25, 1.0, 2.5 and 4.9 mg/kg bw per day. Rats were examined daily for mortality and weekly for clinical signs. Body weight and feed consumption were recorded weekly during the study. Blood and urine were collected from 10 males and 10 females per group at weeks 13, 26, 52, 79 and 105 for haematological examinations, serum biochemical determinations and urinalyses. Ophthalmological examinations were performed before the test and in week 102/103. A complete necropsy was performed on all rats. Selected organ weights of 10 rats of each sex per dose were measured. A large range of tissues was histologically examined.

Mortality in all dose groups, including controls, was high (62–76%, not dose dependent). The numbers of surviving animals in the control, low-, mid- and high-dose groups were 31, 18, 23 and 28 for males and 49, 27, 27 and 23 for females, respectively. In high-dose males, increase incidences of sores on the base of the hind feet (32% control, 64% high dose), prolapsed penis (11% control, 25%

high dose), urine staining (23% control, 35% high dose), alopecia (17% control, 31% high dose) and lethargy (7% control, 15% high dose) were observed. In high-dose females, increased incidences of alopecia (9% control, 24% high dose), urine staining (29% control, 40% high dose) and unkempt appearance (9% control, 17% high dose) were observed. Body weight gain was markedly increased throughout most of the study in females of the mid-dose group (up to 18%) and throughout the entire study in females of the high-dose group (up to 20%). At termination, the body weight of females in the mid-dose group (539 g) was similar to that of controls (528 g), whereas the body weight of high-dose females was still considerably higher (610 g). In high-dose males, a small increase in body weight gain (up to 4%) was observed in weeks 0–52, whereas in weeks 53–85, the growth rate was markedly decreased, resulting in a lower terminal body weight (430 g) compared with control males (body weight 533 g). In the mid-dose males, the body weight gain was increased, in particular during the 2nd year of treatment (up to 16%), although the terminal body weight in mid-dose males (770 g) was similar to that of control males (762 g). Differences in feed consumption between the groups correlated with differences in body weight. Concentrations of erythrocytes, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin and per cent haematocrit were decreased (5–13%) in week 79 in high-dose males. High-dose males had an increased (~50%) concentration of polymorphonuclear leukocytes in weeks 79 and 105, and high-dose females showed an 8-fold increase in eosinophil concentration in week 105. This might be related to the increased incidence of inflammations in kidneys and urinary bladder (see below). Potassium (females and males), calcium (females) and phosphorus (females) levels were slightly increased in high-dose rats in week 105, which might be due to chronic nephritis (see below). Serum triglyceride concentrations were decreased (half of control concentration) in high-dose males and increased (1.5- to 6-fold higher than controls) in mid- and high-dose females in weeks 26, 52 and 79 and in high-dose females in week 105, which correlates with the observed changes in body weight and feed consumption. Cholesterol concentrations were decreased (32–37%) in mid- and high-dose males in weeks 52 and 79 compared with control males. No treatment-induced changes were observed in the urinalyses or ophthalmological analysis. The incidences of organ- and tissue-specific neoplasms were comparable between control and treated rats; differences in percentages of tumour-bearing rats were small and non-significant. In the low-, mid- and high-dose males, dose-dependent increases in relative (to body weight) liver weight (113%, 127% and 150% of controls, respectively) and kidney weight (108%, 127% and 131% of controls, respectively) were observed, which were not accompanied by histopathological changes. Histological examination revealed an increase in plantar granuloma (related to increased foot sores) in high-dose males. Vacuolar degeneration of neurons was observed in the high-dose group in the brain (32 females, 62 males) and spinal cord (4 females, 28 males), but not in the other groups. Other microscopic findings, such as an increase in chronic nephritis in high-dose females and increased chronic proliferative cystitis in the urine bladder of high-dose males, are considered not directly related to treatment.

The NOAEL in this study was 0.25 mg/kg bw per day, based on treatment-related increased body weight gain, triglyceride concentrations in females and relative kidney weight in males at 1.0 mg/kg bw per day (Lankas, 1994d).

The Meeting noted that although mortality in all dose groups, including controls, was high (62–76% at the end of the study, not dose dependent), the numbers of surviving rats are considered sufficient for an evaluation of the carcinogenic potential of emamectin. No differences in tumour development were observed between control and treated rats.

2.4 Genotoxicity

Emamectin (benzoate and hydrochloride) was tested for genotoxicity in a range of guideline-compliant assays. No evidence for genotoxicity was observed in any test.

Table 11. Overview of genotoxicity tests with emamectin[a]

End-point	Test object	Concentration	Purity (%)	Results	Reference
In vitro					
Point mutations	*Salmonella typhimurium* strains TA97a, TA98, TA100, TA1535; *Escherichia coli* WP2, WP2 *uvrA*, WP2 *uvrA* pKM101	2–953 µg/plate (±S9)	92.8 MAB1a, 4.1 MAB1b	Negative	Lankas (1992c)[b]
Chromosomal aberrations	Chinese hamster ovary cells	1–250 µmol/l (±S9, dose range finding), 1–10 µmol/l (±S9, chromosomal aberrations study)	92.5 MAB1a, 5.3 MAB1b	Negative	Galloway (1993a)[c]
Gene mutation	V79 Chinese hamster lung cells, HPRT test	0.005–0.06 mmol/l (+S9), 0.005–0.04 mmol/l (−S9)	96.9	Negative	Lankas (1992d)[d]
DNA strand breaks	Rat hepatocytes	0.003–0.02 mmol/l	92.8	Negative	Lankas (1992e)[e]
In vivo					
Chromosomal aberrations	Male mouse (Crl:CD1) bone marrow (females used for dose range finding)	8, 26 or 80 mg/kg bw (single gavage doses)	95.9	Negative	Galloway (1993b)[f]

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

[a] Positive and negative (solvent) controls were included in all studies.

[b] Emamectin hydrochloride (L-656,748-010V003). Performed in accordance with OECD Test Guideline 471.

[c] Emamectin benzoate (L-656,748-052S002). Two chromosomal aberration tests were performed. The first study gave negative results but was considered not valid because of possible contamination of the dimethyl sulfoxide solvent. Conclusions were based on the second study. Cytotoxicity was observed at high doses. Performed in accordance with OECD Test Guideline 473.

[d] Emamectin hydrochloride (L-656,748-010V003). Precipitation at ≥ 1 mmol/l. Cytotoxicity was observed. Performed in accordance with OECD Test Guideline 476.

[e] DNA strand breaks were measured by the alkaline elution rat hepatocyte assay, described by Bradley & Sins (1984). No OECD guideline exists for this assay. Hepatocytes were isolated from male Sprague-Dawley rats and exposed to emamectin hydrochloride (L-656,748-010V003) dissolved in dimethyl sulfoxide. A range-finding study with a 3-hour exposure was performed to determine cytotoxic levels, determined by trypan blue exclusion. The rat hepatocyte assay was performed twice because of equivocal results; an increase in elution slope was observed at 0.01 mmol/l (76% relative cell viability). However, trypan blue exclusion is a measure of membrane integrity, which is lost in a late phase of toxic cell death. The slight increase in elution slope at 1 mmol/l might therefore be explained by early cytotoxicity.

[f] Emamectin benzoate (L-656,748-052S002). Performed in accordance with OECD Test Guideline 475. Mortality (1 mouse, 80 mg/kg bw) and clinical signs were observed in the chromosomal aberration study. In a range-finding experiment reported in this study, clinical signs were reported at doses of 26 mg/kg bw and higher (see section 2.1).

The results of the genotoxicity tests are summarized in Table 11. It is concluded that emamectin is unlikely to be genotoxic.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

In an oral range-finding reproduction study, mated female Crl:CD(SD)BR rats (12 per group) were administered emamectin benzoate salt (L-656,748-038W002, purity 94.2%) by gavage (dissolved in water) at 0, 0.1, 0.7 and 5.0 mg/kg bw per day or by diet at 0, 1, 7 and 50 ppm (equal to 0, 0.10, 0.67 and 4.6 mg/kg bw per day) from day 1 of gestation through day 20 of lactation. Observations for mortality, clinical signs and parturition were performed daily. Feed consumption and body weight were recorded frequently (~4-day periods). Maternal rats were killed and necropsied at the

end of lactation. The uterus was examined to count metrial glands, and gross examination of the target organs (brain, spinal cord and sciatic nerves) was performed in the gavage groups. Histopathology was performed on the target organs from maternal rats in the control and high-dose gavage groups and on all observed gross lesions. Females that did not deliver were sacrificed at presumed gestation day (GD) 24, and their uteri were examined. First-generation (F_1) pups were sexed and examined for malformations. On day 4, litters were culled to four pups of each sex per litter. Gross necropsy of target organs was performed on F_1 pups (one pup of each sex per litter, 10 litters) at day 21 of age. Histopathology was done on high-dose (diet), mid-dose (diet and gavage) and control (diet and gavage) F_1 pups. Statistical analyses (trend analyses) were performed with data on litter size, body weight and feed consumption.

No mortality or treatment-related clinical signs were seen in maternal rats. Rats treated with 5.0 mg/kg bw per day were euthanized earlier than planned (on lactation days [LDs] 8–15) due to high pup mortality. Body weight gain over GDs 0–4 in the mid- and high-dose gavage groups was 32 and 34 g, respectively, compared with 24 g in the control dams. Dams of the high-dose diet group gained 32 g over GDs 0–4, compared with 19 g in the diet control group. A decrease in body weight gain was found in the high-dose gavage group at GDs 8–16 (42 g versus 56 g in controls) and LDs 0–8 (9 g versus 27 g in controls) and in the high-dose diet group at GDs 8–12 (19 g versus 24 g in controls) and LDs 8–12 (5 g versus 15 g in controls). All these changes in body weight gain were statistically significant ($P \leq 0.05$). Feed consumption showed increases and decreases during gestation and lactation that correlated with changes in body weight gain. There were no treatment-related effects on number of pregnant rats, pregnant rats with living pups or length of gestation during the gestation period or on metrial gland count in the uterus. No treatment-related histological changes or changes in brain weight were observed in the maternal rats. In the high-dose gavage group, death of pups was observed during postnatal days (PNDs) 1–4 (16 pups) and PNDs 5–21 (60/96 pups, $P \leq 0.05$). In the mid-dose group, three and four pups died during PNDs 1–4 and PNDs 5–21, respectively; these deaths were not considered to be treatment related. One pup died in the gavage control group, and none in the low-dose gavage group. No treatment-related increases in deaths of pups were observed in the diet groups. Tremors were observed in the high-dose gavage group (19 pups, PNDs 12–14) and high-dose diet group (64 pups, PNDs 11–21) and are considered treatment related. Other physical signs observed only in the high-dose diet or gavage group were coldness to touch (two pups), weakness (two pups) and paleness, shallow breathing and ring-like constriction on the tail (one pup each). The weight of pups was lower in the high-dose gavage group compared with the control group during the whole postnatal period (5% decrease at PND 0, up to 62% decrease at PND 14, $P \leq 0.05$) and also lower in the high-dose diet group (33–52% decrease, PNDs 14–21, $P \leq 0.05$). In the high-dose diet group, absolute brain weights of F_1 pups were decreased (16–18%), whereas relative brain weights were increased (73–97%). This can be explained by the decreased body weight of these pups compared with control pups. Microscopic examination of pups revealed neuron degeneration in the brain (7/8 males, 5/8 females) and in the spinal cord (7/8 males, 5/8 females) in the high-dose diet group (high-dose gavage group not examined). This degeneration was characterized by swollen neurons within the pons (increased amount of eosinophilic cytoplasm, central chromatolysis, nucleus displacement) and swollen spinal grey matter. Lesions were absent in the other groups examined.

In this range-finding study, no maternal or offspring toxicity was observed at 0.7 mg/kg bw per day. At 4.6–5 mg/kg bw per day (gavage and dietary treatment), decreased body weight gain and reduced feed consumption were observed in the dams. At these doses, mortality, decreased weight gain, tremors and neuron degeneration were observed in the offspring (Wise, 1992).

In a two-generation dietary reproduction study performed according to OECD Test Guideline 416, Sprague-Dawley rats (33 of each sex per group) were fed emamectin benzoate (L-656,748-052S002,

purity > 96%). Dietary concentrations were adjusted to maintain the desired dose levels of 0, 0.1, 0.6 and 3.6 mg/kg bw per day. The actual mean emamectin doses were 0, 0.1, 0.6 and 3.7 mg/kg bw per day. The high dose for F_0 females (following second cohabitation) and for F_{1a} females was reduced to 1.8 mg/kg bw per day (equal to actual mean dose) at GD 0 based on pup effects in the F_1 generation (described below). Parental (F_0) rats were exposed from 9 weeks of age until sacrifice, and F_{1a} rats were exposed from postnatal week 4 until sacrifice. The F_0 generation was mated 2 times. Clinical examination of F_0 rats was performed daily, and body weight and feed consumption were recorded weekly (males, females during premating period) and on GDs 0, 4, 8, 12, 16, 20 and 24 and LDs 0, 4, 8, 12, 16, 20 and 21 (females). Rats were mated in the 9th week of treatment (F_0, first mating) and 3 weeks after weaning of the F_{1a} generation (F_0, second mating). From GD 21 until completion of delivery, each female was observed for parturition on four occasions during weekdays and on two occasions during weekend days. Pups were weighed on PNDs 0, 4, 7, 14 and 21. On PND 4, litters were culled to four pups of each sex per litter, and at PNDs 22–30, one male and one female were selected per litter from 25 litters per dose group. In the adult F_{1a} generation, body weight and feed consumption during gestation and lactation were recorded (see parental rats). F_{1a} rats were mated at postnatal week 19 (treatment week 15). 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 (treatment weeks 24–27) and on F_1 rats (treatment weeks 21–24). The uterus was examined for metrial glands. Testes, epididymis, prostate, seminal vesicles, ovaries, uterus, vagina, brain, spinal cord and sciatic nerve of rats in the control and high-dose groups of all generations and all gross lesions were histologically examined. In addition, brain and spinal cord from the mid-dose group (F_0 and F_{1a}) and sciatic nerve from the low- and mid-dose groups (F_0) were histologically examined. F_{1b} and F_2 rats were examined only for clinical abnormalities, body weight and death of pups.

Mortalities in dams observed in different treatment groups were considered incidental due to their isolated nature. Clinical signs observed in the F_0 generation were equally distributed over the groups and were not treatment related. The average body weight gain of high-dose F_0 females compared with controls was slightly, but statistically significantly, increased (up to 5%) before first mating and during gestation for F_{1b} mating. The high-dose F_0 males showed a slight, but statistically significant, decrease in body weight gain before first mating and during and after the second cohabitation (up to 7%) compared with the control group. Feed consumption in the high-dose F_0 females was increased during the premating periods (up to 15%) and decreased during the first and second lactation periods (13–28% decrease). Other occasionally observed changes in feed consumption were considered not treatment related because a dose–response relationship was lacking. No changes in feed consumption of males were observed. During the first mating (for F_{1a}), the fecundity index (ratio of pregnant females to mated females, in %) was lower in the low-, mid- and high-dose groups (71–76%) compared with controls (91%); the fertility index (ratio of pregnant females to females cohabited, in %) was decreased in a similar manner. During the second mating (F_{1b}), the fecundity index and fertility index were lower only in the high-dose (1.8 mg/kg bw per day) group (74% and 70%, respectively) compared with controls (88% and 85%, respectively). No effect on fecundity was observed in the low- and mid-dose groups during the second mating or during F_{1a} mating (see below). Therefore, reduced fecundity in the low- and mid-dose groups during first mating is considered not treatment related. Infertility of F_0 females (number of females without pregnancy during the first and second mating periods) was increased in the high-dose group (seven females high-dose group, one female control, $P \leq 0.05$).

There was no treatment-related effect on pup survival in the F_{1a} or F_{1b} generation. Several treatment-related clinical signs were observed only in the high-dose group (3.6 mg/kg bw per day) of F_{1a} pups, which included hindlimb extension, hindlimb splay, limited use of hindlimb, head tremors and whole-body tremors (each sign observed in 18–21 of the 22 litters). The incidence of clinical signs in the F_{1b} pups was lower; hindlimb extension, limited use of hindlimbs and whole-body tremors were found (each sign found in 5–7 of the 23 litters) in the high-dose (1.8 mg/kg bw per day) group.

Clinical signs of neurotoxicity were observed first on PND 7. Clinical signs were not observed in the other groups. Male and female F_{1a} pup weight gain was decreased at PND 14 (9–10%) and PND 21 (29–33%) compared with controls ($P \leq 0.05$). No treatment-related physical anomalies were found in F_1 pups. In each control and treatment group of adult F_{1a} rats (males and females combined), two rats died during the study (found dead or sacrificed because of no surviving pups), without relation to treatment. Treatment-related clinical signs observed in the high-dose F_{1a} males and females during the post-weaning period were hindlimb splay (25/25 males, 27/27 females) and whole-body tremors (10/25 males, 13/27 females). Furthermore, alopecia was observed in high-dose adult F_{1a} females during post-weaning (6/27 females), gestation (7/23 females) and lactation (5/11 females) and in adult F_{1a} males of the mid-dose group (4/25 males) and high-dose group (3/25 males) during the post-weaning period. Body weight gain of high-dose F_{1a} males was decreased before cohabitation (−7%) compared with controls ($P \leq 0.05$). Feed consumption during the study was comparable among all groups. The fecundity index (52%) and fertility index (48%) were significantly ($P \leq 0.05$) decreased in the F_{1a} females of the high-dose group, compared with the controls (80%). Mating of the F_{1a} females resulted in 11 females with live pups in the high-dose group and 19 or 20 females with live pups in the other groups. Other reproductive performance parameters were not changed by treatment. No treatment-related pup deaths were observed in the F_2 generation. In one high-dose litter of the F_2 generation, whole-body tremors were observed in all pups, and in the same litter, some pups displayed hindlimb extension. Male pup weight gain of the F_2 high-dose group was decreased (10%) on PND 4. Male and female pup weight gain in the high-dose F_2 group was slightly decreased on PND 14 (5–6%) and PND 21 (7–9% lower weight) compared with control pups. No treatment-related gross lesions were found in rats from the F_0 or F_{1a} generation. Histopathology revealed neuron degeneration in the brain (29/33 males; 23/33 females), neurodegeneration in the spinal cord (31/33 males; 5/33 females) and degeneration of the sciatic nerve (4/33 males) in the F_0 generation high-dose group. These changes were absent in all other examined groups. Neuron degeneration was also observed in the high-dose group of the F_{1a} generation in the brain (23/25 males; 18/27 females) and in the spinal cord (23/25 males; 7/27 females). No treatment-related microscopic changes of the reproductive system were found.

The NOAEL for parental toxicity was 0.6 mg/kg bw per day, based on decreased body weight gain, decreased feed consumption and neuron degeneration observed in the 1.8/3.6 mg/kg bw per day group. The NOAEL for offspring toxicity was 0.6 mg/kg bw per day, based on clinical signs of neurotoxicity, decreased body weight gain and neuron degeneration in the 1.8/3.6 mg/kg bw per day group. The NOAEL for reproductive toxicity was 0.6 mg/kg bw per day, based on decreased fecundity at 1.8/3.6 mg/kg bw per day (Lankas, 1993).

(b) Developmental toxicity

Rats

In a developmental toxicity study performed according to OECD Test Guideline 414, groups of 25 mated female Sprague-Dawley rats were treated orally, by gavage, with emamectin benzoate salt (L-656,748-038W002, purity 94.2%) in deionized water at a dose level of 0, 2, 4 or 8 mg/kg bw from days 6 through 19 of gestation (day 0 = day of observation of copulatory plug). All rats were observed for clinical signs pre-test and daily during the study. Body weight was recorded at day 0 and every other day from day 6. Feed consumption was measured over 2-day periods. All females were killed on day 20 of gestation by carbon dioxide asphyxiation. The uterus was examined, the numbers of corpora lutea and implants were counted and gross examination of thoracic and abdominal viscera was performed. All fetuses were weighed and examined externally. The viscera and coronal head sections were examined in one half of the fetuses per litter and all externally malformed fetuses. Skeletal examination was performed on all fetuses.

There were no deaths or abortions during the study. Tremors (15/25 females) were observed during GDs 10–20, and unkempt coat (3/25 females), few or no faeces (2/25 females) and convulsion (2/25 females) were observed during GDs 16–20 in the high-dose group. These clinical signs were all absent in the other groups, and other observed signs were not treatment related. During GDs 6–14, body weight gain was increased (not statistically significantly) in the low-dose group (51 g) and mid-dose group (48 g) compared with the control group (42 g), whereas the high-dose group had a statistically significantly decreased body weight gain during this period (30 g). During GDs 14–20, body weight gain was statistically significantly decreased in the mid-dose group (80 g) and the high-dose group (60 g) compared with the control group (92 g). Over the entire treatment period (GDs 6–20), the mid- and high-dose groups showed a decreased body weight gain (128 and 90 g, respectively) compared with the control group (135 g). The decreases were statistically significant, as found by trend analyses. Changes in feed consumption showed a trend similar to that for the changes in body weight gain, except for an increase in feed consumption in the high-dose group measured at days 8 and 11 (body weight gain was decreased). The percentage of resorptions of all implants was increased in the high-dose group (4.3% high dose, 1.1% controls). The study authors reported that this high-dose value was close to the mean value of 4.0% found in contemporary historical control groups. However, historical control data were not presented in the study report. Fetal weight was slightly decreased in the high-dose group (4–6%). External and visceral examination of fetuses did not show any treatment-related effects. The number of fetuses with skeletal variations, mainly supernumerary ribs and wavy ribs, was doubled in the high-dose group compared with controls (77/332 high dose, 37/344 controls). Incomplete ossification was observed in twice as many fetuses in the high-dose group as in the controls (14% high dose, 6% controls) and at more sites (e.g. pelvic bone, cervical vertebra, skull bone). A very slight increase in incomplete ossification was also observed in the mid-dose group (9%) compared with controls (6%), with more sites of incomplete ossification. These changes probably relate to the decreased body weight gain and feed intake of the parental females. There were no macroscopic changes related to treatment in the parental females. The results for number of corpora lutea were not given.

The NOAEL for maternal toxicity of emamectin was 2 mg/kg bw per day, based on a decrease in body weight gain at 4 mg/kg bw per day during GDs 14–20. The NOAEL for fetal toxicity was 4 mg/kg bw per day, based on the increased number of resorptions, decreased fetal weight and an increased number of fetuses with skeletal variations and incomplete ossification at 8 mg/kg bw per day (Manson, 1992b).

Rabbits

An oral developmental toxicity study was performed according to OECD Test Guideline 414 with New Zealand White rabbits (18 per group) exposed to emamectin benzoate salt (L-656,748-038W002, purity 94.2%) by an oral catheter at a dose of 0, 1.5, 3 or 6 mg/kg bw per day. Rabbits were artificially inseminated and administered once daily with emamectin from GD 6 to GD 18. All rabbits were observed daily for clinical signs. Body weights were recorded pre-test, every other day during the exposure period and at GDs 19, 20 and 28. Feed consumption was measured every 3rd day. Rabbits were killed on GD 28 by intravenous injection. The uterus was examined to determine the reproductive status (corpora lutea count, implantation count), and gross examination of thoracic and abdominal viscera was performed on all rabbits. Implants were weighed and examined externally (visceral examination, coronal sections of head, skeletal examination).

None of the females died. One female (control group) aborted on GD 19. Clinical examination showed mydriasis (9/18 rabbits) and decreased pupillary reaction (16/18 rabbits) in the high-dose group only. Other clinical signs were unrelated to treatment. Body weight gain in the high-dose group was decreased during GDs 12–19 (−52%) and GDs 19–28 (−15%) compared with control rabbits. The overall body weight gain (GDs 6–28) was decreased in the high-dose group (−28%). Feed consumption was slightly decreased in the high-dose group at GDs 10, 25 and 28. No treatment-related

effects on body weight or feed consumption were observed in the low- and mid-dose groups. No treatment-related gross lesions in maternal rabbits or effects on number of implants or fetus survival were observed. No data were given relating to the number of corpora lutea. Mean fetal weight was increased by 9–13% in the mid-dose group compared with the control group. As the mean fetal weights in the other groups were similar, the increase in the mid-dose group is not considered treatment related. No treatment-related external, visceral or skeletal anomalies were observed in the fetuses.

The NOAEL for maternal toxicity in rabbits was 3 mg/kg bw per day, based on clinical signs and decreased body weight gain at 6 mg/kg bw per day. The NOAEL for fetal toxicity in rabbits was 6 mg/kg bw per day, the highest dose tested (Manson, 1992c).

2.6 Special studies

(a) Neurotoxicity

Mice

A 15-day dietary neurotoxicity study was performed in CF-1 mice, which are known to be highly sensitive to avermectins as a result of their deficiency in mdr1a p-glycoprotein. Mice (51 days of age) were exposed to 0, 0.3, 0.6, 1.8 or 5.4 ppm (equal to 0, 0.06, 0.11, 0.32 and 0.98 mg/kg bw per day for males and 0, 0.06, 0.12, 0.37 and 1.06 mg/kg bw per day for females) emamectin benzoate salt (L-656,748-038W002, purity 96.9%) for 15 days via diet (10 mice of each sex per group). Body weight and feed consumption were recorded weekly. The mice were observed daily for mortality and clinical signs. All mice were necropsied on day 15, and brain, spinal cord and sciatic nerves were examined grossly and microscopically. Brain weights were recorded.

Four mice (one male, three females) were sacrificed in the 5.4 ppm group at 24 hours, day 3 or day 4, and four mice (three males, one female) were sacrificed in the 1.8 ppm group between day 6 and day 15, as a result of their moribund condition. Clinical signs of toxicity, including tremors, ptosis, decreased motor activity, slow righting reflex, urine staining, bradypnoea, splayed hindlimbs and hunched appearance, were observed in six mice (four males, two females) at 1.8 ppm (days 2–4) and in two females at 5.4 ppm (most starting between days 3 and 11). Tremors were observed in one male (sacrificed at 24 hours) in the 5.4 ppm group. No clinical signs were observed in the other groups. The mean body weight gain of females was comparable among all groups. One female in the 1.8 ppm group had a 7.5 g body weight loss between the start and end of the study. The mean body weight gain in males was lower in the 1.8 ppm group (0.6 g) than in the control group (3.4 g). This was mainly due to two males that lost 3.0 or 9.8 g body weight between the pre-test measurement and day 15. The mean feed consumptions were comparable among the groups. No gross lesions or histological changes were observed.

The NOAEL was 0.6 ppm (equal to 0.11 mg/kg bw per day), based on mortality, clinical signs and reduced body weight gain at 1.8 ppm (equal to 0.34 mg/kg bw per day). The increased sensitivity of this mouse model in comparison with other animal models should be taken into account when comparing effect levels (Gerson, 1992d).

In a 2-week dietary neurotoxicity study, male and female Crl:CD-1 mice (10 of each sex per group) were fed emamectin hydrochloride salt (L-656,748-010V003, purity 96.9%) for 13 days. Dietary concentrations were adjusted weekly to achieve the required doses of 0, 0.2, 0.6, 1.2 and 2.0 mg/kg bw per day. Actual compound consumption was 0, 0.2, 0.5, 1.0 and 1.7 mg/kg bw per day. Mice were observed daily for mortality and clinical signs. Body weight and feed consumption were measured weekly. All mice were necropsied on day 14, and the brain, spinal cord and sciatic nerves were examined grossly and microscopically.

No mortality or clinical signs were observed. The total increase in body weight gain over the treatment period in the 0.6 and 1.2 mg/kg bw per day males (6.1 and 8 g, respectively) was higher than that observed in the male control group (4.7 g). The increase occurred in week 1; in week 2, body weight gain was decreased compared with controls. It should be remarked that the mean body weight measured pre-test was lower in the male 1.2 mg/kg bw per day group (21.6 g) than in male controls (24.2 g). The observed increase in body weight gain in males was not confirmed by data from a 13-week dietary range-finding study (Gerson, 1992b) and a 79-week carcinogenicity study (Lankas, 1994c) using the same strain of mice. Body weight gains in females and feed consumption in males and females were similar in all groups. No gross lesions were observed. Microscopic examination revealed cellular infiltration of the meninges of one male in the highest-dose group, but this was considered incidental to treatment.

The NOAEL was 1.7 mg/kg bw per day, the highest dose tested (Lankas, 1992f).

Rats

An acute oral neurotoxicity study was performed, in accordance with OECD Test Guideline 424, in Crl:CD(SD)BR rats treated with emamectin hydrochloride salt (L-656,748-010V003, purity 96.9%) at doses of 0, 27.4, 54.8 and 82.2 mg/kg bw. Rats (10 of each sex per group) were given a single dose via gastric intubation at approximately 6 weeks of age. Mortality and clinical signs were observed daily up to 21 days post-dosing. Body weights were recorded pre-test and on days 7, 14 and 21. At termination of the study at day 21 (exsanguination), gross and histological examinations of the brain, spinal cord, sciatic nerve and optic nerve were performed, and the brains were weighed.

Treatment-related mortality was observed during days 2–6. In the high-dose group, 5 rats (4 males, 1 female) were found dead, and 10 rats (4 males, 6 females) were killed prematurely due to their moribund condition. In the mid-dose group, two males were found dead and two females were killed prematurely for similar reasons. There was no mortality in the other groups. Clinical signs observed included salivation (within 5–30 minutes of dosing), tremors, ataxia, bradypnoea and reddish coloured discharge (within 5–22 hours of dosing) and irritability and decreased activity on days 2–5 that persisted for no more than 1 week. All these clinical signs were observed in the mid- and high-dose groups, and each rat showed at least one of these signs. Tremors, ataxia and/or salivation were observed in 16 of 20 rats in the low-dose group. Other clinical signs included loss of righting reflex, urine staining, hypothermia, ptosis, moist stools and hyperactivity and were found mainly in the mid- and high-dose groups, but also in the low-dose group. No physical signs were seen in the control group. Body weight gain in treatment groups (4–47% males, 18–36% females) was dose-dependently decreased compared with the control group (65% males, 45% females) during the 1st week after exposure. In the remaining 2 weeks, the weight gain was higher in all treatment groups compared with the control group, although differences were smaller than in week 1. Body weight gain during the whole study was 12–17% lower in males and females in the mid-dose group and 12–39% lower in the high-dose group (males, only two rats left) compared with the control group. No gross changes were observed in the planned or prematurely killed rats, despite the observed clinical signs. Brain weights were comparable among all groups. Histological examination revealed degeneration of the white matter in the brains and spinal cord and degeneration of the sciatic nerve in the low-, mid- and high-dose groups (Table 12). Of the seven rats found dead (mainly high-dose group), six had autolysis of the brains and spinal cord, which precluded histological examination.

The lowest-observed-adverse-effect level (LOAEL) was 27.4 mg/kg bw per day, the lowest dose tested, based on clinical signs, decreased body weight gain and histological changes in the brain, spinal cord and sciatic nerve. The calculated acute oral LD_{50} in this study is 67 mg/kg bw in males and 70 mg/kg bw in females (Manson, 1992d).

A second acute oral neurotoxicity study was performed in male and female Crl:CD(SD)BR rats treated with emamectin benzoate salt (L-656,748-038W002, purity 94.2%) at doses of 0, 0.5,

Table 12. Histological treatment-related findings in rats after a single dose of emamectin

	Dose (mg/kg bw)							
	0 (controls)		27.4		54.8		82.2	
	M	F	M	F	M	F	M	F
Number necropsied	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*
Degeneration, brain, white matter	0	0	6	5	7	4	5	3
Degeneration, spinal cord, white matter	0	0	8	5	9	8	5	6
Degeneration, sciatic nerve	2	0	8	7	8	8	2	4

From Manson (1992c)
F, female; M, male

2.5, 5.0, 10 and 25 mg/kg bw. Rats (10 rats of each sex per group) were treated at 6 weeks of age with a single dose of emamectin by gastric intubation. Rats were observed daily for mortality and clinical signs. Body weights were recorded pretreatment and on days 8 and 16 after treatment. Rats were killed at day 22, brain weights were recorded and brains, spinal cord, sciatic nerves and optic nerves were examined grossly and microscopically.

Two female rats, one from each of the 0.5 and 2.5 mg/kg bw groups, were sacrificed prematurely at day 12 owing to weight loss and poor condition. Treatment-related clinical signs were observed in the 10 and 25 mg/kg bw dose groups. All males and females treated with 25 mg/kg bw showed tremors (6 hours after dosing), and most of the animals of this dose group showed irritability (6 hours after dosing), which lasted at most until day 5. Two males and two females in the 10 mg/kg bw group showed tremors, and two of them (one male, one female) also showed irritability (only 6 hours after dosing). Other clinical signs (ataxia, bradypnoea, decreased activity, reddish coloured discharge) were observed in only one or two rats, were not dependent on dose and are considered incidental to treatment. Body weight gains at day 16 were 11% lower in both sexes of the 25 mg/kg bw group compared with the control group. Other treatment groups showed body weight gain similar to that in the control group. No gross lesions were observed. Brain weights were similar in all groups. Histology revealed degeneration of the white matter in the brain (5 males, 7 females) and spinal cord (10 males, 8 females) at 25 mg/kg bw, which was not observed in the other groups. Degeneration of the sciatic nerve was observed at 25 mg/kg bw (10 males, 9 females), at 10 mg/kg bw (2 males, 1 female), at 5.0 mg/kg bw (2 males, 1 female), at 0.5 mg/kg bw (2 males, 2 females) and in the control group (2 males). The increased incidence of sciatic nerve degeneration in the highest-dose group was considered treatment related. The degeneration in the brain was characterized by the presence of vacuoles, which sometimes contained debris or macrophages.

The NOAEL was 5.0 mg/kg bw, based on tremors and irritability observed at 10 mg/kg bw (Manson, 1992e).

A 14-week dietary neurotoxicity study was performed in rats according to OECD Test Guideline 424 (deviation: no ophthalmological examination was performed). Sprague-Dawley (Crl:CD(SD) BR) rats (10 rats of each sex per group) were treated via diet with emamectin benzoate hydrate (L-656,748-052S002, purity 95.9%) for 91–92 days. The concentration of the compound in the feed was adjusted weekly to approximate the required doses of 0, 0.25, 1.0 and 5.0 mg/kg bw per day. Actual compound consumption was 0, 0.24, 0.95 and 4.74 mg/kg bw per day. An additional group of 10 females was included to obtain additional historical control data for FOB observations. All rats were observed daily for mortality and clinical signs. Body weights and feed consumption were recorded once per week. FOB and motor activity tests were conducted pre-test and in weeks 5, 9 and 13/14. The central and peripheral nervous systems, optic nerve and skeletal muscle of selected rats (6 rats

of each sex in control, low-dose and high-dose groups, 10 rats of each sex in mid-dose group) and all gross lesions were sampled for microscopic examination.

No mortality was observed during the study. Treatment-related clinical signs were observed in 8 of 10 males in the high-dose group (body tremors, salivation, slightly soiled, urine staining) in weeks 7–14 and in 2 females in the high-dose group (body tremors) in weeks 11–13. Other signs were not treatment related. The average body weight gain in the high-dose males was 25% lower than that in controls. The decrease in weight gain was observed starting in week 7, and in weeks 12 and 13, weight loss was observed. Weight gain of males in the low- and mid-dose groups was not affected by treatment. Female weight gain was also comparable among all groups. Feed consumption was decreased in weeks 12 and 13 (~5 g less than in week 11) in the male high-dose group, which correlates with the loss of body weight. The results of the FOB revealed signs of neurotoxicity in males of the high-dose group in weeks 9 and 13/14. Effects were found on the central nervous system index (tremors, soiled fur, decreased rearing, flattened position), autonomic nervous system index (salivation) and muscle tone and equilibrium index (body drags, abnormal gait score, impaired mobility, reduced forelimb and hindlimb strength, reduced righting reflex). These three indices were statistically significantly altered in high-dose males in weeks 9 and 13/14 ($P \leq 0.05$). In females of the high-dose group, the forelimb grip strength was reduced in weeks 9 and 13/14 ($P \leq 0.05$), and one female showed mild tremors in week 13/14. No effects were found in the low- and mid-dose groups. Motor activity (horizontal activity) was comparable among all groups. Histological examination showed treatment-related signs in the brain, spinal cord, sciatic nerve and skeletal muscle in rats of the high-dose group (Table 13). Histomorphological findings in the low- and mid-dose groups were considered incidental and not treatment related.

The NOAEL in this study was 0.95 mg/kg bw per day, based on clinical neurotoxicity signs, decreased weight gain (males only), reduced feed consumption, effects on FOB and histological alterations in brain, spinal cord, sciatic nerve and skeletal muscle in the male and female high-dose groups (Gerson, 1992e).

An oral developmental neurotoxicity study was performed according to OECD Test Guideline 426 (deviation: no data for feed consumption). Emamectin benzoate hydrate (L-656,748-052S002, purity > 97%) at dose levels of 0, 0.1, 0.6 and 3.6 mg/kg bw per day was given daily by oral gavage to three groups of 25 female Sprague-Dawley rats (Crl:CD(SD)BR). The high dose level (3.6 mg/kg bw per day) was reduced to 2.5 mg/kg bw per day between GD 17 and GD 20 due to tremors in pups after treatment with emamectin at 3.6 mg/kg bw per day in a concurrent study (see section 2.5, study by Lankas, 1993). Pregnant females were given emamectin from GD 6 to LD 20. Parental (F_0) females were observed daily for clinical signs and mortality. Pupillary reflex was evaluated once per week. Body weights were measured every other day during gestation and twice per week during lactation. Females were terminated at LDs 23–24, the uteri were examined for metrial gland count, and the rats were discarded without further examination. The first-generation (F_1) pups were counted, examined externally, weighed and sexed. On PND 4, litters were culled to four pups of each sex per litter, and three or four rats of each sex per group were selected on PNDs 23–24. The F_1 rats were observed daily for mortality. Physical signs were observed daily during lactation and weekly thereafter. Body weights were recorded on PNDs 0, 4, 11, 17 and 21 during lactation and then weekly thereafter. One rat of each sex per litter was examined 2–4 times in behavioural tests from PND 13 to PND 60. Behavioural tests included motor activity (horizontal activity, stereotypic behaviour), auditory startle habituation (contraction of the muscles as a response to an intense sensory stimulus) and passive avoidance (learning assessment). On PND 11, brains of at least 10 rats of each sex per group were removed and weighed, and six brains of each sex per group were used for histology. Vaginal canalization and preputial separation were recorded as being present 3 times between PND 31 and PND 47. On PND 60, brains of at least 10 rats of each sex per group were removed and weighed. In

Table 13. Histological treatment-related findings in rats after 14 weeks of dietary exposure to emamectin

	Dose (mg/kg bw per day)							
	0		0.24		0.95		4.74	
	M	F	M	F	M	F	M	F
Number necropsied	*6*	*6*	*6*	*6*	*10*	*10*	*6*	*6*
Cytoplasmic vacuolation of neurons, brain	0	0	0	0	0	0	6	6
Cytoplasmic vacuolation of neurons, spinal cord	0	0	0	0	0	0	6	6
Degeneration, spinal cord, white matter	1	1	0	0	1	0	6	4
Degeneration, sciatic nerve	1	0	0	0	1	0	6	1
Atrophy, skeletal muscle	0	0	0	0	0	0	3[a]	0
Chronic focal myositis, skeletal muscle	0	0	1	0	0	0	0	1[b]

From Gerson (1992e)
F, female; M, male
[a] Seven rats observed.
[b] Eight rats observed.

an additional group of six rats of each sex per group, brain, spinal cord, optic nerve, sciatic nerve and skeletal muscle were histologically examined.

No mortality or treatment-related physical signs were observed in the dams during the study. Body weight gain was increased in the mid- (3%) and high-dose groups (6%) during gestation. Over this treatment period, the weight gains in the control, mid-dose and high-dose groups were 111, 123 and 128 g, respectively. Body weight changes during lactation were comparable among all groups. There were no treatment-related changes in reproductive performance (number of pregnant females, length of gestation, pup mortality at birth, number of implants). There was no treatment-related effect on mortality or on external malformations of F_1 rats. Clinical signs were observed in the F_1 high-dose group. They started with intermittent head tremors (10/25 litters) and intermittent body tremors (23/25 litters) during PNDs 6–13 and progressed to whole-body tremors (25/25 litters) observed until PND 27. In addition, hindlimb extension (25/25 litters) was observed during PNDs 10–23, and hindlimb splay (25/25 litters) during PNDs 10–26. Hindlimb splay persisted into the post-weaning period until PND 34. These signs were all absent in the other groups. Other clinical signs were considered incidental. Male and female pup weights in the high-dose group were decreased at PND 11 (10–15%), PND 17 (26–28%) and PND 21 (40–42%), compared with pup weights in the control group. Body weights during the post-weaning period were decreased in males (39% at post-weaning week 1; 21% at post-weaning week 7) and females (43% at post-weaning week 1; 25% at post-weaning week 7) in the high-dose group compared with controls. Motor activity (horizontal activity) was increased at PND 13 ($P \leq 0.05$) in the high-dose group (males plus females). This can be explained by the increase in stereotypy time (time in which stereotypic behaviour is exhibited) observed in the high-dose groups at PND 13, which is probably related to the tremors observed. Motor activity was decreased in the male and female high-dose groups ($P \leq 0.05$) at PND 17 compared with the controls. Activities were comparable at PND 21, but again slightly decreased in the female high-dose group at PNDs 58–60. Motor activity was also decreased in the male and female mid-dose groups (females $P \leq 0.05$) at PND 17 compared with the controls; however, values were within the range of historical control values. The sensorimotor reflexes were decreased in males and females of the high-dose groups. The peak amplitude of each startle movement was decreased at PND 22 (74% decrease, $P \leq 0.05$) and at PNDs 58–60 (25–53% decrease, $P \leq 0.05$), and the interval between stimulus and peak amplitude increased by 22–33% at PND 22 compared with the control group. There were no treatment-related effects on memory observed in the avoidance test. Development of vaginal canalization was observed in 1%

of the females in the high-dose group at PND 31 and 64% at PND 37, whereas in the control, low-dose and mid-dose groups, these percentages were 32–40% at PND 31 and 99–100% at PND 37. Also, preputial separation was delayed in the high-dose group; 12% at PND 43 and 53% at PND 47 showed preputial separation, whereas in the control, low-dose and mid-dose groups, this was seen in 60–63% at PND 43 and 91–95% at PND 47. Absolute brain weight was slightly decreased at PND 11 (males) and PND 60 (males and females) in the high-dose group, whereas relative brain weight was increased. This can be attributed to the decreased body weight in this group compared with control rats. No treatment-related gross lesions or histomorphological changes were observed at PND 11 or PND 60.

The NOAEL for maternal toxicity was 0.6 mg/kg bw per day, based on the increase in body weight gain during gestation observed at 2.5 mg/kg bw per day. The NOAEL for offspring toxicity was 0.6 mg/kg bw per day, based on clinical signs, reduced body weight gain, decreased motor activity and sensorimotor reflexes and delayed development in the high-dose group (Wise, 1993).

Rabbits

An acute dermal neurotoxicity study was performed by treatment of groups of five female New Zealand White rabbits with emamectin benzoate (L-656,748-038W lot No. 2, purity 94.2%) at a dose of 500, 1000 or 2000 mg/kg bw under semi-occluded conditions for 4 or 24 hours. A control group without emamectin exposure was not included. Dry emamectin was applied to an area of shaved skin measuring 10 cm × 10 cm on the backs of each rabbit and moistened with saline. The application site was covered with a gauze patch, wrapped with plastic and removed after 4 or 24 hours. Rabbits were observed for clinical signs 1 and 6 hours after removal of the patches and daily thereafter for 14 days. The application sites were examined immediately after exposure (4 or 24 hours) and daily thereafter. Rabbits were weighed weekly and killed 14 days after dosing. Brain weights were recorded, and brain, spinal cord, a peripheral nerve and all gross lesions were examined histologically.

One rabbit from the high-dose group treated for 24 hours was killed on day 7 due to its moribund condition. This rabbit showed physical signs (tremors, mydriasis, ataxia, decreased activity, moist stools, salivation and weakness) and histological changes (degeneration of neurons and white matter) due to neurotoxicity. After 4 hours of treatment, two females (one low dose, one high dose) showed tremors, and all five females in the high-dose group developed mydriasis on days 3–5. No clinical signs were seen in the mid-dose group. After 24 hours of treatment, tremors and mydriasis were observed in almost all rabbits of all groups, starting at days 3–4 and lasting for several days. In addition, sluggish pupillary response to light was observed on days 3–4 in several rabbits in the mid- and high-dose groups following 24 hours of treatment, and one high-dose rabbit showed soft stools after 24 hours of treatment. Occasionally, erythema was observed in some animals. However, the incidence and severity of the erythema were not dependent on the dose.

Body weights were similar in all groups treated for 4 hours and were constant during the study. The groups treated for 24 hours showed small decreases in body weights at day 14 compared with their pre-test weight (2–4% decrease in the low- and mid-dose groups and 11% decrease in the high-dose group). No treatment-related gross lesions or changes in brain weight were observed. Neuron degeneration and/or degeneration of the white matter were observed in the brain, spinal cord and peripheral nerve in all dose groups after 4 or 24 hours of treatment (Table 14).

A NOAEL could not be determined, as clinical signs, skin lesions, loss of body weight and histological findings in the brain, spinal cord and peripheral nerve were observed at all dose levels (Bagdon, 1992).

Dogs

In a 5-week exploratory neurotoxicity study, male and female Beagle dogs were administered emamectin hydrochloride (L-656,748-010V003, purity 96.9%) by gavage at a dose of 0 (one of each

Table 14. Histological treatment-related findings in rabbits after a single dermal dose of emamectin

	4 h treatment			24 h treatment		
	Dose (mg/kg bw)					
	500	1000	2000	500	1000	2000
Total number necropsied	*5*	*5*	*5*	*5*	*5*	*5*
Degeneration, brain, white matter	0	1	2	3	5	5
Neuron degeneration, brain	0	0	1	3	5	5
Degeneration, spinal cord, white matter	3	0	1	3	4	5
Neuron degeneration, spinal cord	0	0	0	0	0	1
Degeneration, peripheral nerve	1	2	4	4	5	5

From Bagdon (1992)

sex), 0.5 (two of each sex) or 1.5 (three of each sex) mg/kg bw per day. Dogs were examined daily for clinical signs and mortality. Body weights were recorded weekly. Feed consumption was measured 3 times per week. Dogs were killed at different time points: one control male and one dog of each sex per dose group were sacrificed in week 2, one dog of each sex from the 1.5 mg/kg bw per day group was sacrificed in week 3 and all remaining dogs (one control, two low dose, two high dose) were sacrificed in week 5. Necropsy was performed on brain, spinal cord and sciatic nerves, and these organs were microscopically examined.

One male dog in the high-dose group was sacrificed prematurely in week 4 owing to its moribund condition, showing signs of ataxia, tremors, mydriasis, salivation and loss of body weight. Clinical signs (tremors, mydriasis, ptyalism) were observed only in the high-dose group, beginning in weeks 2–3. The duration of the signs tended to increase as the study progressed. Body weights in the high-dose group decreased during the treatment period. The average body weight decrease in week 2 was 0.8 kg (7%). Feed consumption in the high-dose animals was also reported to be reduced. However, no feed consumption data were presented. No gross lesions were observed. Histological examination in the high-dose group showed degeneration of neurons in the brain and spinal cord (two males, two females), degeneration of the white matter in the brain (two males, one female) and spinal cord (two males, two females) and neuron degeneration of the peripheral nerve (two males, one female). No lesions were found in the control or low-dose group. The number of dogs in this study was insufficient for determination of a NOAEL (Lankas, 1992g).

3. Observations in humans

No information was available.

Comments

Studies were performed with emamectin benzoate, unless stated otherwise.

Biochemical aspects

After administration of a single oral dose (0.5 mg/kg bw) of emamectin to rats, maximum concentrations in blood and plasma were reached after 4–12 hours, and emamectin was eliminated with plasma half-lives of 20–51 hours. Comparison of AUC values following oral and intravenous dosing indicated that the oral absorption of emamectin was 55–74%. There is no consistent evidence

for a sex difference in oral absorption. The radiolabel was widely distributed to the tissues, with the highest levels in small intestine, caecum, spleen, liver, lung and adrenals at 3 hours and in pituitary gland, adrenal glands, sublingual glands, Harderian glands, large intestine and lung at 24 hours. The lowest concentrations were found in the brain and spinal cord. Excretion occurred predominantly through faeces, most between 24 and 48 hours after dosing. After 2–3 days, more than 90% of the administered dose of 0.5 mg/kg bw was excreted. Following oral or intravenous administration, emamectin was excreted via bile (2–17%), and only a very small amount was excreted in urine (~1%); the major and remaining portion was excreted in the faeces through efflux transportation into the intestinal tract. Intestinal secretion of emamectin is the main route of elimination, which is consistent with the known role of p-glycoprotein as an efflux transporter of avermectins. Following a single oral dose of 20 mg/kg bw, the maximum concentrations in blood and plasma were reached after 11–15 hours and were approximately 40 times higher than those observed after a single low dose. Also in these high-dose rats, radioactivity levels in other tissues were 40–100 times higher and excretion from the body took 2 days longer in comparison with the low-dose rats. Therefore, these kinetic parameters indicate dose proportionality. Tissue distribution and the proportions eliminated by different routes were similar following the single low and high doses. Administration with a repeated low dose of 0.5 mg/kg bw per day for 14 days resulted in similar kinetics as compared with a single low dose. A steady state with a maximum concentration in plasma 2 times higher than after a single low dose was reached at the seventh dose. Tissue distribution after the repeated low dose was similar to that observed after a single low dose, but with 2 times higher radioactivity levels. Comparison of blood kinetics and excretion between the benzoate hydrate and benzoate salt and between the benzoate salt and hydrochloride salt in dogs indicates that they have similar absorption and kinetics in the blood and similar route and rates of excretion. One metabolite, AB1a, is formed by *N*-demethylation of emamectin and accounts for up to 30% of the administered dose.

Toxicological data

The LD_{50} values for emamectin benzoate dissolved in carboxymethylcellulose were 53–237 mg/kg bw in two rat studies. The LD_{50} for emamectin benzoate hydrate dissolved in carboxymethylcellulose was 58 mg/kg bw. LD_{50} values for emamectin hydrochloride, using water as vehicle, were 67–88 mg/kg bw in two rat studies. Signs of toxicity at high doses included ptosis, hypoactivity, tremors, ataxia, salivation, irritability, bradypnoea, diarrhoea, anogenital staining, reduced faecal volume and weight loss. The LD_{50} for dermal toxicity was 500–2000 mg/kg bw in rats, and the 4-hour acute inhalation LC_{50} was 0.663 mg/l in female rats, but greater than 1.049 mg/l in male rats. Emamectin was slightly irritating to the skin and moderately irritating to the eye of rabbits. Emamectin was not a skin sensitizer in a local lymph node assay in mice.

The primary effect of emamectin was neurotoxicity, as observed in acute neurotoxicity studies in rats and in short-term toxicity studies in rats, rabbits and dogs. In a 13-week dietary range-finding study and a 14-day dietary neurotoxicity study in mice, no signs of neurotoxicity were observed at doses up to 15 mg/kg bw per day.

In a 90-day dietary study with emamectin hydrochloride in rats, the NOAEL was 0.5 mg/kg bw per day, based on cytoplasmic vacuolation of neurons in the brain observed in males at 2.5 mg/kg bw per day.

In a 14-week gavage study with emamectin hydrochloride and a 1-year oral (gavage) study in dogs, the overall NOAEL was 0.25 mg/kg bw per day, based on histological changes in the brain, spinal cord and sciatic nerve and clinical signs of neurotoxicity at 0.5 mg/kg bw per day.

In several studies, changes in body weight gain and feed consumption were observed. A decrease in body weight gain was observed in mice, rats and dogs at doses equal to or greater than 5.0, 2.5 and 0.75 mg/kg bw, respectively. However, increased body weight gain was also observed in

several studies in rats at 1.0–2.5 mg/kg bw per day. Such increases in body weight gain have been observed previously upon treatment with ivermectin and are generally characteristic of avermectins. As the mechanism by which avermectins increase body weight gain is unknown, the Meeting considered that this effect should be considered potentially adverse and could not be disregarded.

In a 1-year dietary study in rats, the NOAEL was 0.1 mg/kg bw per day, based on increased body weight gain observed at 1.0 mg/kg bw per day.

In a 79-week study in mice, the NOAEL was 2.5 mg/kg bw per day, based on clinical signs of toxicity and reduced body weight gain observed at 5.1 mg/kg bw per day. No effect of emamectin on tumour incidence was found at doses up to and including 7.6 mg/kg bw per day, the highest dose tested.

In a 2-year study in rats, the NOAEL was 0.25 mg/kg bw per day, based on an increase in body weight gain and increases in triglyceride concentrations in serum and relative kidney weight at 1.0 mg/kg bw per day. No increased incidence of tumours was observed at doses up to 2.5 mg/kg bw per day, the highest dose tested.

The overall NOAEL for the 1-year and 2-year dietary studies in rats was 0.25 mg/kg bw per day, based on the effects observed at 1.0 mg/kg bw per day.

The Meeting concluded that emamectin is not carcinogenic in rodents.

Emamectin was tested in an adequate range of in vitro genotoxicity tests and one in vivo test. No evidence for genotoxicity was observed in any test.

The Meeting concluded that emamectin 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 emamectin is unlikely to pose a carcinogenic risk to humans.

A two-generation study of reproductive toxicity in the rat was performed with doses of 0, 0.1, 0.6 and 3.6 mg/kg bw per day. Based on marked clinical signs of toxicity observed in F_{1a} pups, the high dose was reduced to 1.8 mg/kg bw per day during the course of the study. The NOAEL for parental toxicity was 0.6 mg/kg bw per day, based on decreased body weight gain, decreased feed consumption and neuron degeneration observed at 3.6 (reduced to 1.8) mg/kg bw per day. The NOAEL for reproductive toxicity was 0.6 mg/kg bw per day, based on decreased fecundity at 3.6 (reduced to 1.8) mg/kg bw per day. The NOAEL for offspring toxicity was 0.6 mg/kg bw per day, based on clinical signs of neurotoxicity, decreased body weight gain and neuron degeneration observed at 3.6 (reduced to 1.8) mg/kg bw per day.

Other emamectin-like substances also induce postnatal toxicity in rats over a time period similar to that observed with emamectin. For these closely related compounds, it has been shown that these effects are a direct consequence of low p-glycoprotein levels in the neonatal rat brain and incomplete development of the blood–brain barrier. In the developing human fetus, adult levels of p-glycoprotein expression are attained by about 28 weeks of gestation, reflecting the integrity of the blood–brain barrier prior to birth. Therefore, the Meeting considered that human neonates are less susceptible than neonatal rats to neurotoxicity induced by emamectin. The NOAEL for offspring toxicity established from the study of reproductive toxicity in rats is therefore considered to be sufficiently protective for the developing human fetus and neonate.

In a developmental toxicity study in rats, the NOAEL for maternal toxicity was 2 mg/kg bw per day, based on reduced body weight gain at 4 mg/kg bw per day during GDs 14–20. The NOAEL for fetal toxicity was 4 mg/kg bw per day, based on an increase in fetal resorptions, decreased fetal weight and an increased number of fetuses with skeletal variations and incomplete ossification at 8 mg/kg bw per day.

In a developmental toxicity study in rabbits, the NOAEL for maternal toxicity was 3 mg/kg bw per day, based on clinical signs (mydriasis, decreased pupillary reaction) and decreased body weight

gain from GDs 12 to 28 at 6 mg/kg bw per day. The NOAEL for fetal toxicity in rabbits was 6 mg/kg bw per day, the highest dose tested.

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

In two acute oral (gavage) neurotoxicity studies in rats, one using emamectin hydrochloride and the other using emamectin benzoate, the overall NOAEL was 5.0 mg/kg bw, based on clinical signs of neurotoxicity (tremors and irritability) starting at 10 mg/kg bw. At higher doses, salivation, ataxia, bradypnoea, decreased activity, urine staining, loss of righting reflex, hypothermia, ptosis, moist stools and hyperactivity were observed. Degeneration of the white matter in the brains and spinal cord, degeneration of the sciatic nerve and decreased body weight gain were observed at single doses of 25 mg/kg bw and higher.

In a 14-week dietary neurotoxicity study in rats, emamectin induced clinical signs (body tremors, salivation, slightly soiled, urine staining) and changes in the FOB test (tremors, soiled fur, decreased rearing, salivation, abnormal gait, impaired mobility, reduced limb strength or grip strength, reduced righting reflex) at a dose of 4.74 mg/kg bw per day. These signs were first observed during week 7 of treatment. Histological examination of these rats showed degeneration of neurons and white matter in the brain, spinal cord and sciatic nerve and atrophic skeletal muscles. Furthermore, body weight gain and feed consumption were decreased. The NOAEL in this study was 0.95 mg/kg bw per day.

In a developmental neurotoxicity study in rats using emamectin benzoate hydrate, the NOAEL for maternal toxicity was 0.6 mg/kg bw per day, based on an increase in body weight gain during gestation at 2.5 mg/kg bw per day. The NOAEL for offspring toxicity was 0.6 mg/kg bw per day, based on clinical signs (head tremors, body tremors, hindlimb extension, hindlimb splay), decreased motor activity, decreased sensorimotor reflexes and decreased weight gain observed at 2.5 mg/kg bw per day. Clinical signs of toxicity were not observed until postnatal day 6.

A toxicokinetic study and a 2-week neurotoxicity study were performed with CF-1 mice, which are deficient in expression of p-glycoprotein (*Mdr1a* gene). The toxicokinetic study showed increased emamectin concentrations in the brain and slower excretion rates as compared with wild-type mice. In the 2-week neurotoxicity study, the NOAEL was 0.12 mg/kg bw per day, based on mortality and clinical signs of neurotoxicity at 0.34 mg/kg bw per day. In another 2-week neurotoxicity study, wild-type CD-1 mice did not show mortality or clinical signs of neurotoxicity at 1.7 mg/kg bw, the highest dose tested. The absence of p-glycoprotein has never been shown in humans, and heterozygosity still results in functional p-glycoprotein. The results from CF-1 mice are therefore considered not relevant for human risk assessment. It was previously concluded by WHO that the CF-1 polymorphic mouse is not an appropriate model for human risk assessment for avermectins.

No data on the effects of emamectin in humans were provided.

The Meeting concluded that the existing database on emamectin is sufficient to characterize the potential hazards to fetuses, infants and children.

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) for emamectin benzoate of 0–0.0005 mg/kg bw on the basis of an overall NOAEL of 0.25 mg/kg bw per day in the 1-year and 2-year rat studies, for increased body weight gain, triglyceride concentrations in serum and relative kidney weight at 1.0 mg/kg bw per day, and on the basis of an overall NOAEL of 0.25 mg/kg bw per day in 14- and 53-week toxicity studies in dogs, for histological changes in the brain, spinal cord and sciatic nerve and clinical signs of neurotoxicity at 0.5 mg/kg bw per day, using a safety factor of 500. An additional safety factor of 5 was applied to the default safety factor of 100, as a number of studies in mice, rats and dogs show steep dose–response curves and irreversible histopathological effects in neural tissue at

the LOAEL. A NOAEL based predominantly on such histopathological changes is considered to be less sensitive than the observation of clinical signs. Moreover, in the 1-year dog study, animals were killed in extremis at doses that were only 3 times higher than the NOAEL in this study.

The Meeting established an acute reference dose (ARfD) of 0.03 mg/kg bw for emamectin benzoate, based on a NOAEL of 5 mg/kg bw for clinical signs of neurotoxicity (tremors and irritability) observed in an acute neurotoxicity study in rats at 10 mg/kg bw. A safety factor of 200 was applied, which includes a 2-fold factor based on serious histopathological observations of degeneration of neurons in brain, spinal cord and sciatic nerve at 25 mg/kg bw. Observations of toxicity observed in neonatal rats in reproductive toxicity studies and a developmental neurotoxicity study were considered not relevant for setting an ARfD, as these effects are a direct consequence of low p-glycoprotein levels in the neonatal rat brain and incomplete development of the blood–brain barrier. In the developing human fetus, adult levels of p-glycoprotein expression are attained by about 28 weeks of gestation, reflecting the integrity of the blood–brain barrier prior to birth.

Levels relevant for risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Seventy-nine-week study of toxicity and carcinogenicity[a]	Toxicity	2.5 mg/kg bw per day	5.1 mg/kg bw per day
		Carcinogenicity	5.1 mg/kg bw per day[b]	—
Rat	Fourteen-week study of toxicity[a]	Toxicity	0.5 mg/kg bw per day	2.5 mg/kg bw per day
	One-year study of toxicity and 2-year study of toxicity and carcinogenicity[a]	Toxicity[c]	0.25 mg/kg bw per day	1.0 mg/kg bw per day
		Carcinogenicity	2.5 mg/kg bw per day[b]	—
	Two-generation study of reproductive toxicity[a]	Parental toxicity	0.6 mg/kg bw per day	1.8/3.6 mg/kg bw per day[c]
		Offspring toxicity	0.6 mg/kg bw per day	1.8/3.6 mg/kg bw per day[c]
		Reproductive toxicity	0.6 mg/kg bw per day	1.8/3.6 mg/kg bw per day[c]
	Developmental toxicity study[d]	Maternal toxicity	2 mg/kg bw per day	4 mg/kg bw per day
		Embryo and fetal toxicity	4 mg/kg bw per day	8 mg/kg bw per day
	Acute neurotoxicity studies[e]	Neurotoxicity	5 mg/kg bw	10 mg/kg bw
	Developmental neurotoxicity study[a]	Maternal toxicity	0.6 mg/kg bw per day	2.5 mg/kg bw per day
		Offspring toxicity	0.6 mg/kg bw per day	2.5 mg/kg bw per day
Rabbit	Developmental toxicity study[d]	Maternal toxicity	3 mg/kg bw per day	6 mg/kg bw per day
		Embryo and fetal toxicity	6 mg/kg bw per day[b]	—
Dog	Fourteen-week and 1-year studies of toxicity[d,e]	Toxicity	0.25 mg/kg bw per day	0.5 mg/kg bw per day

[a] Dietary administration. Dietary concentrations of emamectin were adjusted over the course of the study in order to obtain the required daily doses (mg/kg bw per day) for the different dose groups and sexes. Therefore, ppm values are not presented.
[b] Highest dose tested.
[c] Based on marked clinical signs of toxicity observed in F_{1a} pups, the high dose of 3.6 mg/kg bw per day was reduced to 1.8 mg/kg bw per day during the course of the study.
[d] Gavage administration.
[e] Two studies combined.

Estimate of acceptable daily intake for humans

0–0.0005 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 exposures in humans

Critical end-points for setting guidance values for exposure to emamectin benzoate

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	55–74% (rats)
Distribution	Widely distributed (rats)
Potential for accumulation	Low
Rate and extent of excretion	At 0.5 mg/kg bw, > 90% excretion within 72 h, mainly via faeces through efflux transportation from the blood to the intestine; ~1% excretion in urine (rats)
	Plasma half-lives (0.5 mg/kg bw): 20–51 h
	Plasma half-lives (20 mg/kg bw): 35–36 h
Bioequivalence	Emamectin benzoate hydrate, benzoate salt and hydrochloride salt have similar oral absorption, blood kinetics and excretion (dogs)
Metabolism in animals	Limited, metabolism via *N*-demethylation to form the metabolite AB1 (rat)
Toxicologically significant compounds (animals, plants and the environment)	Emamectin benzoate
Acute toxicity	
Rat, LD_{50}, oral	Emamectin benzoate dissolved in carboxymethylcellulose: 53–237 mg/kg bw
	Emamectin benzoate hydrate dissolved in carboxymethylcellulose: 58 mg/kg bw
	Emamectin hydrochloride dissolved in water: 67–88 mg/kg bw
Rat, LD_{50}, dermal	500–2000 mg/kg bw (males), ≥ 1893 mg/kg bw (females)
Rat, LC_{50}, inhalation	> 1.049 mg/l (males), 0.663 mg/l (females)
Rabbit, dermal irritation	Slightly irritating to the skin
Rabbit, ocular irritation	Moderately irritating to the eye
Mice, dermal sensitization (local lymph node assay)	Not sensitizing
Short-term studies of toxicity	
Target/critical effect	Nervous system (clinical signs, lesions in brain, spinal cord, sciatic nerve) (rat, rabbit, dog)
	Body weight increase (rat)
Lowest relevant oral NOAEL	0.25 mg/kg bw per day (dog), 0.1 mg/kg bw per day (rat)
Lowest relevant dermal NOAEL	No data
Lowest relevant inhalation NOAEC	No data
Genotoxicity	
	Not genotoxic

Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Increased body weight gain (both sexes), increased relative kidney weight in males, increased serum triglyceride levels in females (rats)
Lowest relevant NOAEL	0.25 mg/kg bw per day (rat)
Carcinogenicity	Not carcinogenic (mouse, rat)
Reproductive toxicity	
Reproduction target/critical effect	Decreased fecundity
Lowest relevant reproductive NOAEL	0.6 mg/kg bw per day (rat)
Developmental target/critical effect	Decreased fetal weight, increased number of skeletal variations and delayed ossification
Lowest relevant developmental NOAEL	4 mg/kg bw per day (rat)
Neurotoxicity/delayed neurotoxicity	
Acute oral neurotoxicity, NOAEL	5.0 mg/kg bw (rat); tremors, irritability
Acute dermal neurotoxicity, NOAEL	< 500 mg/kg bw (rabbit, lowest dose tested); degeneration of brain, spinal cord, peripheral nerve, tremors, mydriasis
Ninety-day neurotoxicity, NOAEL	0.95 mg/kg bw per day (rat); tremors, degeneration and vacuolation in brain, spinal cord, sciatic nerve
Developmental neurotoxicity, NOAEL	0.6 mg/kg bw per day (rat, offspring); reduced weight gain, tremors, hindlimb extension/splay, decreased motor activity, delayed development of sex organs
Medical data	
	No data

Summary

	Value	Study	Safety factor
ADI	0–0.0005 mg/kg bw	One-year and 2-year studies of toxicity in rats; 14-week and 1-year studies of toxicity in dogs	500
ARfD	0.03 mg/kg bw	Acute neurotoxicity study in rats	200

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ETOFENPROX

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Explanation

Etofenprox, also referred to as ethofenprox, is the International Organization for Standardization (ISO)–approved name for 2-(4-ethoxyphenyl)-2-methylpropyl 3-phenoxybenzyl ether (International Union of Pure and Applied Chemistry), with the Chemical Abstracts Service No. 80844-07-1.

Similar to pyrethroids, etofenprox acts on ion channels of the insect nervous system. It is used as an insecticide with contact and stomach action against many pests on a broad range of crops.

Etofenprox 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 carcinogenicity study in mice and using a 100-fold safety factor. It 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 etofenprox have been submitted: an absorption, distribution, metabolism and excretion study in male rats, acute oral and dermal toxicity studies in rats, a 4-week dermal toxicity study in rabbits, a 4-week dietary mechanistic study on thyroid function and hepatic microsomal enzyme induction in rats, a developmental toxicity study in rabbits, acute and subchronic (90-day) neurotoxicity studies in rats, a developmental neurotoxicity study in rats and 4-week immunotoxicity studies in mice and rats. In addition, oral and dermal acute toxicity studies, a 13-week toxicity study and genotoxicity studies of a plant metabolite of etofenprox, 2-(4-ethoxyphenyl)-2-methylpropyl 3-phenoxybenzoate (α-CO), were conducted.

All critical studies complied with good laboratory practice (GLP).

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

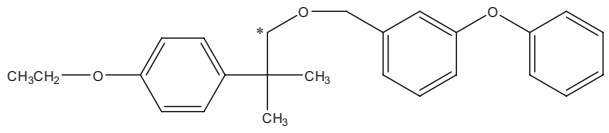
Rats

The absorption, distribution, metabolism and excretion of [^{14}C]etofenprox were investigated (Hawkins et al., 1985a) using a 1:1 mixture of [^{14}C]etofenprox radiolabelled on either side of the ether linkage: [1-^{14}C-propyl]etofenprox (specific activity 566 GBq/mol, radiochemical purity > 99% by thin-layer chromatography [TLC]) and [α-^{14}C-benzyl]etofenprox (specific activity 366 GBq/mol, radiochemical purity 97% by TLC). Only the latter label was used in the studies of Tomoda (1986) and Burri (2001a) (Figure 1).

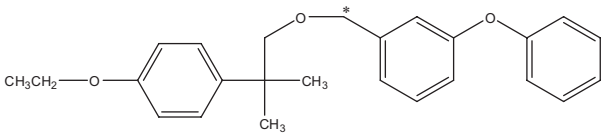
The radiolabelled materials were diluted as appropriate with unlabelled etofenprox (batch No. ST-103, purity not specified). Studies were conducted in 5- to 9-week-old male, non-pregnant female and time-mated female Sprague-Dawley-derived rats (CD strain, approximate weight 200 g, Charles River, United Kingdom). Time-mated female rats were young adults weighing 236–254 g. The rats were treated orally, by gavage, at a constant dose volume of approximately 2.5 ml/kg bw, according to the schedule in Table 1. With the exception of the preliminary study, the doses for all study elements contained both radiolabelled forms, each contributing approximately 50% of the radioactivity.

Preliminary study and excretion–retention study rats were housed in individual metabolism cages for the separate collection of urine and faeces. Expired air was collected only in the preliminary study, because no radioactivity was detected in the expired air traps after dosing with [α-^{14}C-benzyl]etofenprox, and only 0.3% and 0.1% of the dose were recovered after dosing with [1-^{14}C-propyl]etofenprox in males and females, respectively. Qualitative whole-body autoradiographs were obtained from 20 µm sagittal sections at several levels between the kidneys and the spinal cord. Relative tissue concentrations of radioactivity were estimated by visual inspection. The minimum

Figure 1. Structures of the radiolabelled molecules



1-^{14}C-propyl-etofenprox



α-^{14}C-benzyl-etofenprox

detectable levels of radioactivity in the autoradiographs were 1.0–2.7 µg equivalents (eq) per gram wet tissue or 1.5–4.0 µg eq/g wet tissue for placental transfer study rats. Samples of pup stomach contents for milk transfer analysis were obtained according to the schedule in Table 1 by substitution of up to three pups per litter per time point. Naive pups were introduced into the litters of treated dams and allowed to suckle for 1 hour before they were killed and their stomach contents removed. A maternal blood sample was withdrawn at each sampling interval.

The findings of the preliminary study were closely confirmed by the main single oral dose study. The routes and rates of elimination of radioactivity after single oral doses of 30 and 180 mg/kg bw were similar in males and females and at the different dose levels (Table 2). The main route of elimination was via faeces and accounted for 86.4–90.4% of the administered dose in both sexes at both low and high dose levels. Most faecal elimination occurred within 72 hours of administration. Urinary elimination accounted for 6.3–10.7% of the administered dose in both sexes at the low and high dose levels, most of which was eliminated within the first 24 hours after administration. Retention of radioactivity in the body 120 hours after administration was low in both sexes at the low and high dose levels and amounted to 3.4–4.3% of the administered dose.

Biliary excretion of the 30 mg/kg bw dose amounted to 15.2% and 29.6% of the administered dose in males and females, respectively, whereas 9.9% and 10.3% were excreted in the bile of males and females, respectively, receiving the 180 mg/kg bw dose. The rate of biliary excretion was greatest 3–15 hours after treatment in both sexes following administration of the 30 and 180 mg/kg bw doses. Based on biliary excretion, the data suggest that the extent of oral absorption of the 180 mg/kg bw dose would be slightly lower than that at 30 mg/kg bw in intact rats (Table 3).

Faecal elimination, representing unabsorbed dose, amounted to 75.2–77.8% of the administered dose in males receiving 30 mg/kg bw and both sexes receiving 180 mg/kg bw, whereas in females receiving 30 mg/kg bw, faecal elimination accounted for 49.5% of the dose. Urinary elimination in both sexes of bile duct–cannulated rats and at both doses amounted to 1.3–3.3% of the administered doses. The data from bile duct–cannulated rats indicated a greater degree of absorption than indicated by urinary excretion in the intact main-study rats. Furthermore, a substantial proportion of the radioactivity in faecal extracts was associated with metabolites (see below). Thus, a minimum of 54.1% and 53.3% of the administered dose was orally absorbed at 30 mg/kg bw, and 45.8% and 38.1% of the dose at 180 mg/kg bw, in males and females, respectively. Based on urinary elimination, carcass residues and faecal metabolites, the extent of oral absorption is likely to be approximately 64–68% of the dose at 30 mg/kg bw and 48–58% of the dose at 180 mg/kg bw.

Table 1. Treatment schedule and sampling regime used in the absorption, distribution, metabolism and excretion studies of etofenprox in rats

Study element	Dose (mg/kg bw)	No. of doses	Position of radiolabel	No. of rats (M + F)	Sampling regime
Preliminary study	30	1	1-^{14}C-propyl	1 + 1	Urine: 0–8, 8–24, 24–48, 48–72, 72–96 and 96–120 h
	30	1	α-^{14}C-benzyl	1 + 1	Faeces: 24 h intervals for 5 days
					Expired air: 0–48 h
Excretion–retention	30	1	1:1 mixture	5 + 5	Urine: 0–8, 8–24, 24–48, 48–72, 72–96 and 96–120 h
	180	1	1:1 mixture	5 + 5	Faeces: 24 h intervals for 5 days
					Tissues: liver, kidneys, gastrointestinal tract, muscle, fat
Biliary excretion	30	1	1:1 mixture	3[a] + 3[a]	Bile: 3 h intervals for 48 h
	180	1	1:1 mixture	3[a] + 3[a]	Urine: 0–24, 24–48 h
					Faeces: 0–48 h
					Tissues: liver, gastrointestinal tract
Pharmacokinetics	30	1	1:1 mixture	5 + 5	Blood: before dosing and at 0.25, 0.5, 1, 2, 3, 5, 7, 24, 48, 72, 96, 120, 144, 168 and either 192 h (30 mg/kg bw) or 216 h (180 mg/kg bw)
	180	1	1:1 mixture	5 + 5	
Quantitative tissue distribution	30	7	1:1 mixture	25 + 25	5M + 5F killed at 4, 24, 48, 120 and 240 h
					Tissues: blood, liver, kidneys, heart, lungs, fat, brain, eyes, gonads, spleen, pancreas, muscle, adrenals, thymus, thyroid, gastrointestinal tract + contents
Qualitative whole-body autoradiography	30	7	1:1 mixture	5 + 0	1M killed at 4, 24, 48, 120 and 240 h after final dose
		1		1 + 0	1M killed at 24 h
Placental transfer	30	7	1:1 mixture	0 + 10[b]	2F killed at 4, 24, 48, 72 and 120 h after final dose
				0 + 5[b]	Tissues: blood, liver, kidneys, heart, adrenal glands, placentae, fetuses, mammary gland
					1F killed at 4, 24, 48, 72 and 120 h for qualitative whole-body autoradiography
Milk transfer	30	14	1:1 mixture	0 + 3[c]	1–3 pups/dam killed 7 h after 9th, 11th and 14th doses and 1, 2, 3, 5 and 7 days after 14th dose
				0 + 4[d]	
					Pup stomach contents sampled for metabolite profiling
					Maternal plasma taken 7 h after 9th, 11th and 14th doses and 1, 2, 3, 5 and 7 days after 14th dose

From Hawkins et al. (1985a)

F, female; M, male

[a] Restrained rats surgically prepared with bile duct cannulae.

[b] Pregnant females on day 10 of gestation at first dose.

[c] Pregnant females on day 18 of gestation at first dose.

[d] Untreated females at day 18 of gestation for provision of naive pups.

Table 2. Mean elimination of radioactivity after single oral doses of [^{14}C]etofenprox at 30 or 180 mg/kg bw: main study

Matrix	Time (h after dosing)	% of administered dose			
		30 mg/kg bw		180 mg/kg bw	
		Males	Females	Males	Females
Urine	0–8	4.5	2.9	1.8	1.6
	8–24	4.3	3.6	4.3	3.0
	24–48	1.2	0.9	1.4	1.0
	48–72	0.4	0.3	0.4	0.5
	72–96	0.2	0.1	0.1	0.1
	96–120	0.1	0.1	0.1	0.1
	0–120	10.7	7.9	8.1	6.3
Cage wash	120	0.1	0.1	0.1	0.1
Faeces	0–24	38.2	35.7	42.6	45.9
	24–48	37.7	38.4	35.1	19.1
	48–72	7.7	9.6	8.0	16.9
	72–96	3.2	1.6	2.3	7.4
	96–120	1.2	1.1	1.0	1.1
	0–120	88.0	86.4	89.0	90.4
Gastrointestinal tract	120	0.5	0.6	0.4	0.5
Liver	120	0.07	0.04	0.06	0.05
Kidneys	120	0.005	0.004	0.004	0.005
Carcass	120	2.8	2.9	3.8	3.4
Total	0–120	102.2	97.9	101.5	100.7

From Hawkins et al. (1985a)
[a] Including contents.

Table 3. Mean excretion of radioactivity after a single oral dose of etofenprox at 30 or 180 mg/kg bw in bile duct–cannulated animals: biliary excretion study

Matrix	Time (h after dosing)	% of administered dose			
		30 mg/kg bw		180 mg/kg bw	
		Males	Females	Males	Females
Urine[a]	0–48	2.0	3.3	1.4	1.3
Bile	0–48	15.2	29.6	9.9	10.3
Faeces	0–48	75.9	49.5	77.8	75.2
Carcass	48	2.8	5.7	3.0	1.5
Liver	48	0.05	0.2	0.2	0.04
Gastrointestinal tract[b]	48	1.5	9.1	3.3	14.4
Total	48	97.5	97.4	95.6	102.7

From Hawkins et al. (1985a)
[a] Including cage wash.
[b] Including contents.

Mean maximum plasma concentrations (C_{max}) were 5.20 and 5.03 µg eq/ml in males and females, respectively, receiving 30 mg/kg bw and 17.3 and 16.4 µg eq/ml in males and females, respectively, receiving 180 mg/kg bw. These C_{max} values were observed at about 5 hours (range 2–7 hours) after dosing. The plasma concentrations declined rapidly thereafter until they were less than 1 µg eq/ml by 24 hours and 48 hours after administration of 30 and 180 mg/kg bw, respectively. Subsequently, a slower decline was observed after both dose levels until 192 and 216 hours in the 30 and 180 mg/kg bw dose groups, respectively, at which times the plasma concentrations in males receiving 30 mg/kg bw and both sexes receiving 180 mg/kg bw were below the levels of quantification. In females receiving 30 mg/kg bw, a mean residual level of 0.13 µg eq/ml was observed at 192 hours. The areas under the plasma concentration–time curve (AUC) were 93 and 83 µg·h/ml in males and females, respectively, receiving 30 mg/kg bw and 308 and 315 µg·h/ml in males and females, respectively, receiving 180 mg/kg bw. The ratios of AUC values for a dose ratio of 6 were 3.3 and 3.8 in males and females, respectively.

The highest tissue concentrations 120 hours after single doses of 30 or 180 mg/kg bw were observed in fat, where they were about 50-fold higher than the concentrations in liver and at least 100-fold higher than in kidneys and muscle. The tissue concentrations relative to dose level in the four tissues examined approximately reflected the 6-fold difference in the dose levels evaluated, as the relative concentrations varied between 4.2 (kidneys in males) and 8.5 (fat in females).

Following seven daily oral doses of 30 mg/kg bw, tissue concentrations were highest in all tissues of both sexes 4 hours after the last dose (Table 4), at which time adrenal glands, fat, gastrointestinal tract, kidneys, liver, lungs, pancreas, thymus, thyroid gland and ovaries showed higher concentrations than in plasma. Other than the gastrointestinal tract with contents, the highest concentrations (in descending order) occurred in fat, adrenal glands, liver, pancreas, ovaries and thyroid gland. All other tissues showed maximum tissue concentrations at 4 hours that were less than or equal to 8.84 µg eq/g. Concentrations declined rapidly in all tissues except fat and pancreas, in which concentrations declined to 25.0 and 45.2 µg eq/g in fat in males and females (with estimated half-lives of approximately 5 and 8.5 days in males and females) and 8.00 and 12.2 µg eq/g in pancreas in males and females at 240 hours. However, whole-body autoradiography showed the concentrations to be lower in pancreas than in most other tissues, suggesting that the high levels recorded in the quantitative study might have been due to contamination by contiguous fat. In most other tissues, there was a rapid decline in concentration during the first 24 hours after the cessation of treatment, followed by a slower decline to 240 hours. Tissues showing higher concentrations at 240 hours were adrenal glands (1.55 and 5.06 µg eq/g in males and females, respectively), gastrointestinal tract with contents (2.98 and 5.07 µg eq/g in males and females, respectively), thyroid gland (< 1.10 and < 1.00 µg eq/g in males and females, respectively) and ovaries (4.55 µg eq/g). At this time, other tissue concentrations were less than or equal to 0.67 µg eq/g. Comparison of liver, kidney, fat and muscle concentrations 120 hours after one dose or seven daily doses showed that mean radioactivity concentrations were higher after seven doses by a factor of 2.7–5.5, with the exception of female muscle, in which the tissue concentration was 13-fold higher.

The results of qualitative whole-body autoradiography in male rats were generally consistent with the quantitative findings, with the exception of pancreas, as mentioned above. Maximum tissue concentrations occurred 4 hours after the seventh dose and were highest in the gastrointestinal tract, bile ducts and fat, with slightly lower levels in the liver. Relatively low concentrations were observed in the other tissues, specifically in the pancreas. Tissue concentrations declined in all tissues except fat at 24, 48 and 120 hours, with the distribution pattern reflecting the excretion pattern of etofenprox and metabolites. After 240 hours, detectable radioactivity was confined to fat and the lower gastrointestinal tract, although the concentration in fat had declined substantially from the 120-hour concentration.

Table 4. Mean tissue concentrations after seven daily doses of [^{14}C]etofenprox at 30 mg/kg bw: quantitative tissue distribution study

Tissue	Mean tissue concentration (µg eq/g wet weight)									
	Males					Females				
	Hours after seventh dose									
	4	24	48	120	240	4	24	48	120	240
Adrenals	41.4	13.1	5.21	2.94	1.55	43.4	13.0	12.0	5.13	5.06
Brain	2.77	0.23	0.19	0.10	0.06	2.17	0.30	0.20	0.12	0.09
Eyes	1.54	0.24	0.15[a]	0.09[a]	< 0.06	1.06	0.37	0.22	0.17	0.07[a]
Fat	94.2	81.1	78.4	45.0	25.0	101	88.7	86.3	61.0	45.2
Gastrointestinal tract[b]	271	53.0	17.3	9.28	2.98	443	146	38.7	10.5	5.07
Heart	5.00	0.58	0.43	0.24	0.12	4.79	0.88	0.54	0.32	0.18
Kidneys	8.84	1.72	1.31	0.39	0.22	8.71	2.95	1.50	0.83	0.58
Liver	30.5	6.39	3.20	1.45	0.53	22.3	5.33	3.11	1.59	0.55
Lungs	8.20	2.51	2.54	1.25	0.53	7.27	4.51	3.54	1.74	0.67
Muscle	3.50	0.56	2.02	0.21	0.46	2.60	0.64	0.92	0.67	0.52
Ovaries	—	—	—	—	—	23.9	14.4	9.92	5.63	4.55
Pancreas	25.1	22.4	25.0	11.9	8.00	30.8	18.2	15.6	10.8	12.2
Plasma	6.93	0.63	0.34	0.14	0.07	5.39	0.80	0.39	0.17	0.06
Spleen	2.66	0.65	0.84	0.28	0.16	3.14	1.38	1.37	0.53	0.39
Testes	2.79	0.50	0.35	0.26	0.16	—	—	—	—	—
Thymus	7.46	1.84	1.47	0.83	0.44	6.51	4.59	3.72	1.12	0.65
Thyroid	18.7	5.43	4.11	1.67[a]	< 1.10	12.9	8.89	4.72	2.78	< 1.00
Whole blood	4.31	0.44	0.24	0.12	0.07	3.34	0.50	0.25	0.12	0.06

From Hawkins et al. (1985a)

[a] Mean calculated based on assumption that values below limit of accurate detection were equal to the limit.

[b] Including contents.

The concentrations and rates of decline of radioactivity in the adrenal glands, kidneys, heart and liver of pregnant rats (Table 5) were similar to those in non-pregnant females (Table 4). Tissue concentrations, including placentae and fetuses, were highest at 4 hours and declined in all tissues thereafter. The rate of decline in mammary gland was lower than in other tissues, and at 120 hours, 32.4 µg eq/g wet weight tissue remained, a tissue concentration similar to that of fat in non-pregnant females. The calculated half-life for mammary gland was 3.5 days, assuming first-order kinetics. The concentrations in placentae and fetuses at 4 hours were very low relative to plasma concentration, and after 120 hours, the concentrations in placentae and fetuses were comparable to maternal plasma concentrations. Whole-body autoradiography of pregnant animals confirmed the results of the quantitative study, and the general distribution of radioactivity was similar to that in male rats.

Radioactivity was secreted into maternal milk during the treatment period, but decreased markedly on cessation of treatment. Mean concentrations in pup stomach contents 7 hours after dosing during the multiple treatments were in the range 47.9–72.3 µg eq/g, compared with mean maternal plasma concentrations in the range 2.2–3.4 µg eq/ml, giving concentration ratios greater than 20. The concentration in pup stomach contents fell rapidly during the first 31 hours after the cessation of treatment to 1.7 µg eq/g, at which time maternal plasma concentration was 0.4 µg eq/ml. Thereafter, concentrations declined more slowly, giving ratios of concentrations in pup stomach contents

Table 5. Mean tissue concentrations of radioactivity in pregnant rats after seven oral doses of [^{14}C]etofenprox at 30 mg/kg bw: placental transfer study

Tissue	Mean tissue concentration (μg eq/g wet weight)				
	Hours after seventh dose				
	4	24	48	72	120
Adrenal glands	61.5	11.3	12.2	7.88	5.74
Heart	8.03	0.88	1.01	0.52	0.49
Kidneys	9.68	1.99	1.58	1.24	1.09
Liver	27.2	4.57	3.01	2.26	1.55
Mammary gland	87.4	61.9	56.5	43.2	32.4
Fetuses (group 1)	1.72	0.16	0.12	0.14	0.10
Fetuses (group 2)	1.61	0.17	0.19	0.15	0.14
Placentae (group 1)	4.61	0.62	0.37	0.27	0.17
Placentae (group 2)	4.81	0.59	0.37	0.27	0.17
Plasma (μg/ml)	7.05	0.87	0.36	0.25	0.10

From Hawkins et al. (1985a)

to concentrations in plasma of between 4 and 5. Approximately 95% of radioactivity in pup stomach contents was identified as unchanged etofenprox.

Dogs

The absorption, distribution, metabolism and excretion of [^{14}C]etofenprox were investigated in dogs using the same materials as used in the studies described above on rats. Two male and two female Beagle dogs (8–11 months old, weight range 10.3–14.8 kg, Beaulong Ltd, United Kingdom) were treated once orally, by gavage, with a nominal dose of 30 mg/kg bw (actual net doses 25.1–26.7 mg/kg bw) in a volume of 5 ml polyethylene glycol 400 (PEG400), followed by a wash-through with an additional 9 ml PEG400. This dose was used to examine excretion and plasma concentrations. Approximately 4 or 6 months later, one dog of each sex per time point was redosed with [^{14}C]etofenprox 1:1 mixture in the same manner, with net doses in the range 27.2–30.4 mg/kg bw, and used for the determination of tissue distribution.

Radioactivity was eliminated rapidly and predominantly in the faeces, with a mean of 89.5% (range 79.4–96.2%) of the administered dose eliminated by this route over 120 hours; however, a mean of 86.7% (78.1–93.6%) was eliminated during the first 24 hours after administration. Urinary excretion, including cage washes, over 120 hours accounted for a mean of 6.20% (range 4.30–8.56%) of the administered dose, but again most (4.49%, range 2.84–6.73%) was eliminated during the first 24 hours. The rate and routes of elimination were similar in males and females. Faecal elimination was the predominant route of excretion of radioactivity, so the extent of absorption of etofenprox is difficult to estimate from the data. However, as unchanged etofenprox was not eliminated in bile, unchanged etofenprox in faeces is likely to represent unabsorbed material, which amounted to at least 48.5% and 59% in males and females, respectively. The rate of absorption of radioactivity was quite variable, plasma C_{max} being observed 0.25–6 hours after dosing. The group sizes (two of each sex) are probably too small to draw any firm conclusions regarding sex differences, but there was a tendency for absorption to be more rapid in the females. C_{max} occurred at about 0.25 hour in one female and 1–2 hours in the other, compared with about 2 hours in one male and 3–6 hours in the other. Thereafter, plasma concentrations declined, with half-lives in the range 8.6–17.0 hours for first-order kinetics. Maximum concentrations of radioactivity in plasma were within the range 4.43–7.16 μg eq/ml.

The highest concentrations of radioactivity in other tissues were found in the liver (3.1–9.6 µg eq/g wet tissue), followed by kidneys (1.0–3.3 µg eq/g wet tissue), fat (< 0.4–4.1 µg eq/g wet tissue) and muscle (0.3–0.6 µg eq/g wet tissue). These values were dwarfed by the concentrations observed in bile of the male and female sampled (1036 and 815 µg eq/g, respectively), indicating the importance of biliary excretion of radioactivity in dogs (Hawkins et al., 1985b).

1.2 Biotransformation

Rats

TLC of faecal extracts from rats treated with [1-^{14}C-propyl]etofenprox or [α-^{14}C-benzyl]-etofenprox indicated that cleavage of the etofenprox molecule was not a significant metabolic process. The faecal extracts resolved into two major components, one of which was unchanged etofenprox, and a number of minor components. Most unchanged etofenprox was excreted during the first 24 hours after administration and was considered to represent unabsorbed material. The other major component (component 3) was subsequently resolved into two radioactive metabolites (3A and 3B). Desethyletofenprox (3A) occurred at 19.5–25.1% of the dose and was identified by electron impact and chemical ionization mass spectrometry. Etofenprox hydroxylated in the 4′ position of the phenoxybenzyl moiety (3B) occurred at 7.2–13.8% of the dose and was identified by electron impact and chemical ionization mass spectrometry and nuclear magnetic resonance spectrometry. None of the other components of rat faecal extracts were identified. Most of the radioactivity in bile was immobile on TLC, but enzymatic hydrolysis reduced immobility to 5–6%. The major radioactive metabolites released by hydrolysis and separated by TLC were desethyletofenprox and etofenprox hydroxylated in the 4′ position of the phenoxybenzyl moiety. Four minor hydrolysed bile metabolites corresponded to the minor metabolites found in faeces. High-performance liquid chromatographic (HPLC) analysis of fat demonstrated that 94.6% and 93.2% of fat radioactivity in male and female rats, respectively, corresponded to unchanged etofenprox, whereas minor amounts of desethyletofenprox and etofenprox hydroxylated in the 4′ position of the phenoxybenzyl moiety were found. The major identified components in liver extracts were unchanged etofenprox (22.5% and 30.3% in males and females, respectively) and desethyletofenprox (8.1% and 10.3% in males and females, respectively); however, substantial proportions of radioactivity were immobile (43.3% and 24.1% in males and females, respectively), which were considered to represent conjugates (glucuronide and sulfate conjugates). The effect of enzyme hydrolysis was limited to reducing the proportion of immobile radioactivity to, respectively, 31.6% and 18.1% in males and females and to increasing the proportion of desethyletofenprox to, respectively, 16.4% and 24.8% in males and females. The 4′-hydroxylated derivative of etofenprox was not markedly affected by enzyme incubation of liver. Most of the radioactivity in urine was not mobile during TLC, but enzyme incubation released some non-mobile radioactivity to form two unknown metabolites accounting for 1.5% and 2.0% of the administered dose at 30 mg/kg bw and 0.3% and 1.4% of the administered dose at 180 mg/kg bw. Only minor amounts of the metabolites desethyletofenprox and 4′-hydroxyetofenprox were found in urine, and the proportions did not markedly alter on enzyme incubation. There were no significant sex-related differences in the metabolism of etofenprox in any of the matrices examined (Hawkins et al., 1985a).

α-CO is a major metabolite or degradation product isolated during metabolism studies in plants and soil degradation and photodegradation studies, but was not identified in rat and dog metabolism studies (Hawkins et al., 1985a,b). Nevertheless, 3-phenoxybenzyl 2-(4-hydroxyphenyl)-2-methylpropyl ether (DE) and 2-(4-ethoxyphenyl)-2-methylpropyl 3-(4-hydroxyphenoxy)benzyl ether (4′-OH) were identified and are likely degradation products of α-CO. Consequently, the metabolism of etofenprox to α-CO was specifically investigated by Tomada (1986) in a non-GLP study.

The TLC analysis of methanol extracts of the faeces of rats administered [α-^{14}C-benzyl]-etofenprox provided three components (F1, F2, F3) that were extracted with acetone and methanol. The aqueous phase of the untreated urine extracts contained most of the radioactivity, but F1, F2 and F3 were identified in the organic phase. Acid hydrolysis of the aqueous phase released most of the activity into the organic phase as F3. Using prepared reference substances, component F1 was provisionally identified as unchanged etofenprox and F2 as α-CO, and F3 was further resolved into 4′-OH, 3-phenoxybenzoic acid (m-PB-acid) and 3-(4-hydroxyphenoxy) benzoic acid (4′-OH-PB-acid) (Tomada, 1986).

Additional study of the metabolism of etofenprox in rats was undertaken in order to provide evidence for the proposed metabolic pathways (Burri, 2001a). The study was performed to determine the metabolites of [^{14}C]etofenprox in faeces and selected organs and tissues. Unlabelled etofenprox (batch No. 9604, purity > 99%) and [α-^{14}C-benzyl]etofenprox (batch No. MRH/MTC 277/20, specific activity 1.22 GBq/mmol, radiochemical purity > 99% by HPLC) as a solution in ethanol/PEG400 was administered to a group of four fasted (for 16–18 hours) 6- to 9-week-old male Wistar rats (HanBrl:WIST strain, weight range 148–163 g, RCC Ltd, Switzerland). The rats were kept individually in all-glass metabolism cages. Treatment was once orally, by gavage, at a target dose level of 30 mg/kg bw (actual dose 33.5 mg/kg bw) and at a constant dose volume of approximately 5 ml/kg bw. The [α-^{14}C-benzyl]etofenprox was repurified prior to use to 97.7%, but reanalysis before and after administration indicated an average purity of 94.0%.

Urine and faeces were collected from each rat 0–24 and 24–48 hours after dose administration. The rats were killed by asphyxiation with carbon dioxide at 48 hours, and blood was collected from the thoracic cavity after incision of the heart and sampled into heparinized tubes. Heart, liver, lungs, stomach, thymus, spleen, intestinal tract with contents, adrenal glands, kidneys, testes, epididymides, ovaries, uterus, muscle bone (femur), brain, skin, fat, thyroid gland, pancreas and residual carcass were removed and weighed. The cages were washed with acetone/water at the end of the study for the determination of residual radioactivity. Faecal samples from 0–24 hours and 24–48 hours were pooled and extracted with acetonitrile. The extracts were analysed without further processing by HPLC and TLC. Liver samples were pooled, homogenized, extracted under acidic and basic conditions with acetonitrile and subjected to mild acid hydrolysis for 16 hours. Non-extractable radioactivity in faeces and liver was determined following combustion. The first nine liver acetonitrile extracts were combined and partitioned, and the organic and aqueous phases were analysed by TLC and HPLC. Fat samples were pooled, extracted with dichloromethane and partitioned, and the phases were analysed by TLC and HPLC. Urine samples from each 24-hour collection interval were analysed directly by TLC and HPLC. Separated metabolites in the extracts were identified by co-chromatography with authentic reference standards (Table 6).

The mean total excretion up to 48 hours after dosing was approximately 67% of the dose (Table 7), mainly via the faeces (mean 50.4% dose), but also in urine (mean 14.4% dose). Retained radioactivity amounted to 17.3% of the dose, the bulk of which was located in the intestinal tract and carcass. The mean total recovery of radioactivity, including that in cage washes, was 84.3% of the dose. As etofenprox is known to bind to surfaces, a balance of 84.3% may be a reflection of this characteristic. The higher tissue concentrations of radioactivity at 48 hours were found in the intestinal tract (24.2 µg eq/g), fat (16.7 µg eq/g), liver (3.43 µg eq/g), skin (3.00 µg eq/g), epididymides (2.49 µg eq/g), carcass (2.09 µg eq/g) and pancreas (1.94 µg eq/g). In all other tissues, the concentrations were below 1.00 µg eq/g.

In the non-partitioned organic extract of faeces, the 0–24 and 24–48 hour samples contained 13.71% and 36.73% of the administered dose, respectively. In the 0–24 hour sample, unchanged etofenprox occurred at 6.83% of the dose, and three other identified metabolites (4′-OH, DE and 3-hydroxybenzyl 2-(4-ethoxyphenyl)-2-methylpropyl ether [DP]) occurred at 2.76%, 2.12% and 0.71% of the dose, respectively. Seven unidentified fractions occurred at 0.10–0.38% of the dose.

Table 6. Reference standards and abbreviations used for metabolites of etofenprox

Reference material	Abbreviation	Purity (%)
2-(4-Ethoxyphenyl)-2-methylpropyl 3-phenoxybenzoate	α-CO	99.4
3-Phenoxybenzyl 2-(4-hydroxyphenyl)-2-methylpropyl ether	DE	99.6
2-(4-Ethoxyphenyl)-2-methylpropyl alcohol	PENA	98.9
2-(4-Ethoxyphenyl)-2-methylpropionic acid	EPMP	98.1
3-Hydroxybenzyl 2-(4-ethoxyphenyl)-2-methylpropyl ether	DP	90.2
3-Phenoxybenzoic acid	m-PB-acid	98
3-Phenoxybenzyl alcohol	m-PB-alc	98
2-(4-Ethoxyphenyl)-2-methylpropyl 3-(4-hydroxyphenoxy)benzyl ether	4′-OH	78.62
2-(4-Acetoxyphenyl)-2-methylpropyl 3-phenoxybenzyl ether	4-AcO	95.34
2-(4-Hydroxyphenyl)-2-methylpropyl alcohol	OH-Palc	96.0
3-(4-Hydroxyphenoxy) benzoic acid	4′-OH-PB-acid	69.15

From Burri (2001a,b)

Table 7. Balance of radioactivity and excretion of [α-^{14}C-benzyl]etofenprox after one oral dose of 30 mg/kg bw

Matrix	Sampling interval (h post-treatment)	% of radioactivity administered	
		Mean	Standard deviation
Urine	0–24	12.11	2.52
	24–48	2.34	0.74
	0–48	14.45	2.97
Faeces	0–24	13.71	8.09
	24–48	36.73	9.62
	0–48	50.43	11.65
Cage wash	48	2.11	0.97
Total excreted	0–48	66.99	9.27
Fat	48	0.92	0.34
Kidneys	48	0.02	0.01
Liver	48	0.54	0.12
Organs/tissues	48	0.27	0.08
Intestinal tract	48	10.58	6.54
Carcass	48	4.95	1.47
Total retained	48	17.27	8.38
Balance	48	84.26	1.15

From Burri (2001a)

The corresponding 24–48 hour faecal extracts contained a lower proportion of unchanged etofenprox (4.73% of the dose) and higher proportions of both identified and unidentified metabolites. The major metabolite fractions were again 4′-OH, DE and DP, which occurred at 8.84%, 9.17% and 4.65% of the dose, respectively. An additional metabolite (3-phenoxybenzyl alcohol [m-PB-alc]) that was not found in the earlier sample occurred at 0.45% of the dose. Seven unidentified fractions occurred at 0.62–1.72% of the dose.

Neither etofenprox nor α-CO was found in urine, but two identified and four unidentified metabolites were present. The 0–24 hour urine sample contained 12.11% of the dosed radioactivity, but the contributing components were unidentified, apart from m-PB-acid and 4′-OH-PB-acid, which occurred at up to 1.36% and 0.36% of the dose, respectively. A single unknown component occurred at 7.85% of the dose.

Fourteen identified and unidentified metabolites were separated in the organic extract of the liver, in total accounting for 25.9% of the radioactivity recovered from the liver. The identified metabolites were DE, DP, m-PB-acid, m-PB-alc and 4′-OH-PB-acid, each of which accounted for 0.8–1.5% of the recovered dose. Nine unidentified metabolites each occurred at 0.8–7.1% of the recovered dose. None of the three components of liver in aqueous extracts were identified, but they accounted for 6.5%, 12.3% and 15.0% of the recovered dose. Unchanged etofenprox (3.9% of the recovered dose) and two unidentified metabolites (0.9% and 2.0% of the recovered dose) occurred in the hexane extract of liver. In the hexane extract of fat, unchanged etofenprox was the only compound detected and accounted for 96.2% of the recovered dose.

This study confirms that excretion of etofenprox by male rats occurs rapidly and predominantly via the faeces, although a small proportion is eliminated in the urine. The putative metabolite α-CO, however, was not detectable in faeces, liver, fat and urine in the Hawkins et al. (1985a) study, although it was later detected in a subsequent study lacking formal identification (Tomada, 1986). Furthermore, the occurrence of m-PB-acid and 4′-OH-PB-acid in liver and urine suggests that α-CO may be a transient metabolite of etofenprox (Burri, 2001a). The fate of α-CO was therefore the subject of a separate, non-GLP investigation (Burri, 2001b), the design of which was similar to that used by Burri (2001a).

Unlabelled α-CO (batch No. 1000821-4, purity 99.74%) and [α-^{14}C-benzyl]α-CO (batch No. CP-2491-1, specific activity 2.03 GBq/mmol, radiochemical purity 99.7% by HPLC) as solutions in ethanol/PEG400 were used for the study. The purity of the ^{14}C-labelled material was found to be, on average, 99.7% before and after administration.

The mean total excretion up to 48 hours after dosing was approximately 99.8% of the dose, mainly via the urine (73.76%), but an additional 14.80% of the dose was found in faeces. Both urinary and faecal elimination were rapid, the proportions of the dose over 0–24 hours being 69.1% in urine and 11.57% in faeces. The cage washes contained 11.24% of the dose. Retained radioactivity amounted to 1.0% of the dose, the bulk of which was located in the intestinal tract and carcass. Therefore, [^{14}C]α-CO was rapidly and almost completely eliminated from the body. All tissue concentrations at 48 hours were very low, the higher values occurring in the intestinal tract (1.300 µg eq/g), kidneys (0.480 µg eq/g), liver (0.339 µg eq/g), blood plasma (0.297 µg eq/g) and epididymides (0.266 µg eq/g). In all other tissues, the concentrations were below 0.200 µg eq/g. No parent α-CO was detected in urine up to 48 hours after treatment, but seven radioactive fractions were separated, most of which were unidentified. The major metabolite fractions after 0–24 hours were unknown U7 (40.28% of the dose), unknown U6 (15.69% of the dose) and m-PB-acid (8.43% of the dose). The two major but unknown metabolites are considered to represent small metabolites or conjugates of α-CO and other metabolites. The other separated metabolites, 4′-OH-PB-acid and three additional unidentified fractions, occurred at 0.0–2.63% of the dose. Unidentified metabolite U7 occurred at 3.29% of the dose at 25–48 hours, and others occurred at 0.0–0.80% of the dose. In the non-partitioned organic extract of faeces, the 0–24 and 24–48 hour samples contained 10.38% and 2.76% of the administered dose, respectively. In the 0–24 hour sample, unchanged α-CO occurred at 3.86% of the dose, together with m-PB-acid at 1.19% of the dose, 4′-OH-PB-acid at 0.96% of the dose and six other unidentified metabolites that occurred at 0.10–1.92% of the dose. The corresponding 24–48 hour faecal extracts contained no unchanged parent compound, but there were low concentrations of m-PB-acid (0.43% of the dose), 4′-OH-PB-acid (1.49% of the dose) and two unidentified fractions, together accounting for 0.84% of the dose.

This study demonstrated that α-CO is rapidly and almost completely eliminated from the body, predominantly in the urine; little residual α-CO remains in the tissues after 48 hours; and elimination is mainly as parent compound, m-PB-acid, 4′-OH-PB-acid and two very polar unidentified metabolites.

On the basis of these studies, the metabolic pathway shown in Figure 2 was proposed.

Dogs

TLC of faecal extracts obtained during the oral administration investigations described above demonstrated the presence of one major radioactive component co-chromatographing with etofenprox. Electron impact and chemical ionization mass spectrometry confirmed the identity of unchanged etofenprox, which accounted for 91.4% and 93.3% of extracted radioactivity, equivalent to 48.5% and 59.0% of the administered dose, in the 0–24 hour samples of males and females, respectively. Other components occurred in very small quantities, the largest of which was component 3 at 6.1% and 4.6% of extracted radioactivity, equivalent to 3.5% and 2.9% of the administered dose, in males and females, respectively. Component 2 was present at 1.1% and 0.8% of extracted radioactivity, equivalent to 0.6% and 0.5% of the administered dose, in males and females, respectively. These same components were found in bile. All other mobile components together accounted for 0.4% and 0.2% of extracted radioactivity.

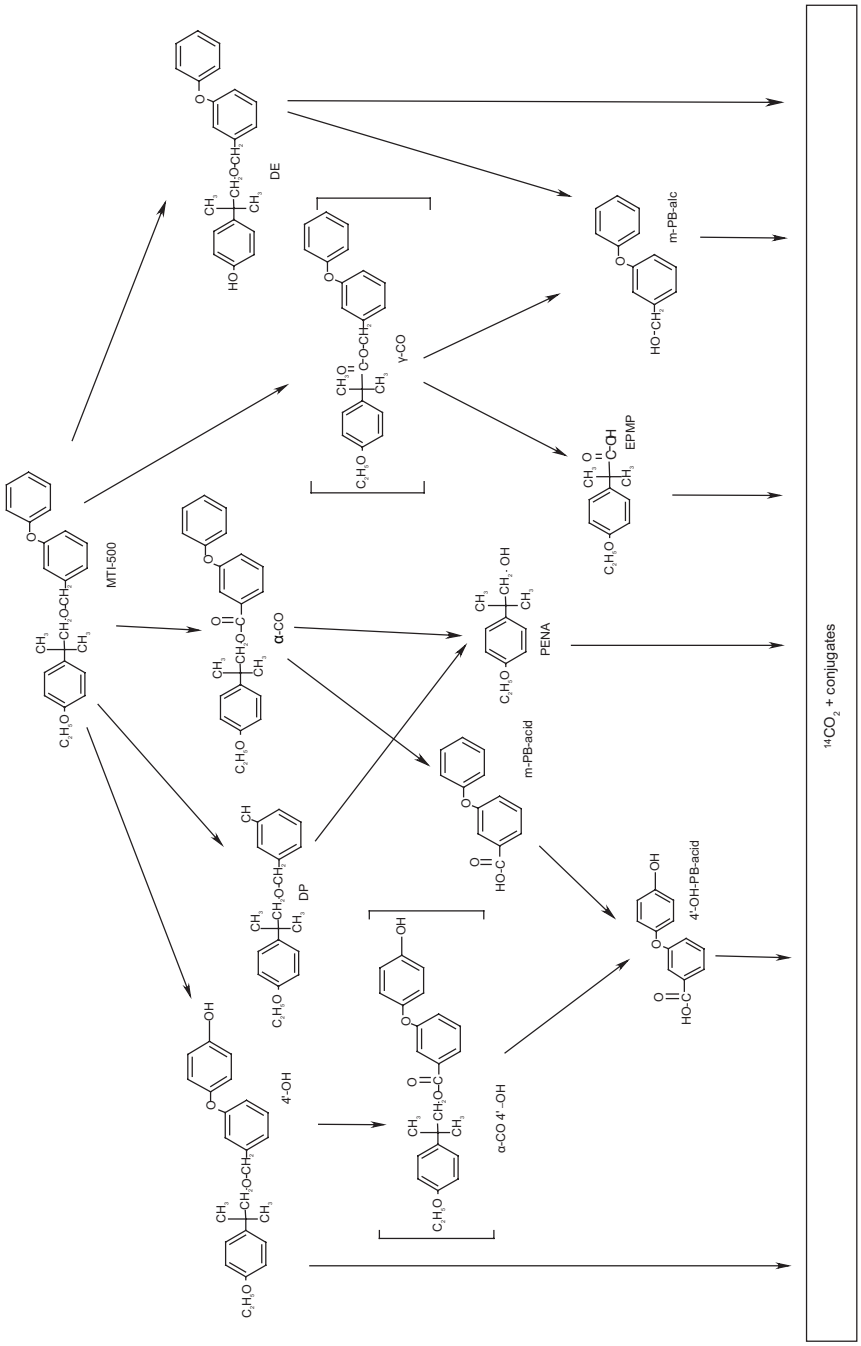
Almost all (97.7%) of the radioactivity in bile was non-mobile during TLC, suggesting the presence of polar conjugated metabolites. Enzymatic hydrolysis released one major component (40.5% and 37.3% in males and females, respectively) and three minor components comprising 3.3–6.1% of the radioactivity in bile. Acid hydrolysis reduced non-mobile radioactivity further to 8.1% and released a spectrum of components similar to that found after enzyme treatment. However, there were greater relative proportions of the more polar minor metabolites. The major metabolite fraction 3 was subsequently resolved into fractions 3A and 3B, accounting for 14.5% and 28.8% of the radioactivity in the bile, respectively. The major metabolite fractions 3A and 3B and the minor fraction 2 corresponded to the same fractions in faecal extracts.

Urine collected during the first 24 hours after treatment contained 4.49% of the administered dose. The patterns of radioactive components showed little variation between the sexes, and enzymatic hydrolysis had almost no effect. Components 3A and 3B together accounted for 1.2–1.7% of the administered dose, and non-mobile radioactivity accounted for up to 2.2% of the administered dose. Other minor fractions accounted for up to 0.8% of the administered dose in untreated and enzyme-hydrolysed urine.

The faecal extracts resolved into one major component and a number of minor components. Unchanged etofenprox was identified by electron impact and chemical ionization mass spectrometry as the major component. Component 3 was resolved into two radioactive metabolites. Component 3A was identified by electron impact mass spectrometry as desethyletofenprox (derived by *O*-deethylation of the ethoxyphenyl group), with supporting evidence from the chemical ionization spectrum. Component 3B was identified by electron impact and chemical ionization mass spectrometry as 4′-hydroxyetofenprox (derived by ring hydroxylation of the phenoxybenzyl moiety). None of the other components of faecal extracts were identified.

Analysis of the radioactive material in fat demonstrated that more than 80% of the extracted radioactivity co-eluted with etofenprox using HPLC. Assuming that this identification is correct, the calculated tissue concentrations were 3.0 and 2.5 µg eq/g in male and female dogs, respectively. Etofenprox accounted for 11% and 18% of liver radioactivity, equivalent to 0.6 and 1.7 µg eq/g, in males and females, respectively. Small proportions of the radioactivity in liver corresponded to the faecal and bile components 2 and 3, but most radioactive material was not eluted. After protein precipitation from plasma, radioactivity was quantitatively recovered in the soluble fraction, 25% of which was unchanged etofenprox, equivalent to 0.7 and 0.8 µg/ml in males and females, respectively. More than 60% of the radioactivity did not elute and was assumed to be primarily conjugates of components 3A and 3B (Hawkins et al., 1985b).

Figure 2. Proposed metabolic pathway for etofenprox in rats (see Table 6 above for names of metabolites)



Source: Burri (2001a)

2. Toxicological studies

2.1 Acute toxicity

Studies of the acute toxicity of etofenprox are summarized in Table 8.

(a) Oral administration

Mice

Five-week-old ICR mice (10 of each sex per dose) were treated with a single gavage dose of etofenprox (batch Nos ST-101 and ST-102, purity 96%) at 0, 50 or 100 ml/kg bw (equivalent to 0, 53.6 and 107.2 g/kg bw, respectively). Mortality, clinical signs and body weights were recorded for 14 days. All mice were necropsied and selected tissues (not specified) taken for microscopic examination.

One male at 50 ml/kg bw died on day 5, and one female in each of the 50 and 100 ml/kg bw groups died on days 1 and 3, respectively. Treatment-related clinical signs (comprising watery diarrhoea within 15–20 minutes of treatment, soiled fur, tails lifted up, an apparent temporary increase in body temperature and increased respiration rate at both dose levels, yellowish soft faeces occurring at 24 hours accompanied by anal prolapse in some animals, abdominal swelling and facial oedema) were observed. All clinical signs subsided in survivors within 5 days of treatment, and body weight gain was not affected by treatment. No treatment-related gross findings were observed in decedents or survivors. The oral median lethal dose (LD_{50}) was greater than 107.2 g/kg bw in both sexes (Hashimoto, 1982b).

Rats

Two groups of 7-week-old male and female Sprague-Dawley rats (Crj:CD(SD)IGS) (five of each sex per dose) were given etofenprox (batch No. 20024, purity 99.0%) as a single gavage dose of 0 (vehicle) or 2000 mg/kg bw suspended in corn oil (10 ml/kg bw).

No deaths or clinical signs of toxicity were observed during the 14-day observation period. No gross pathology was observed at necropsy. Etofenprox produced a slight depression of body weight gain during the first 24 hours following treatment in both sexes. The oral LD_{50} of etofenprox in rats was greater than 2000 mg/kg bw in both sexes (Oda, 2003a).

Groups of 5-week-old Sprague-Dawley-derived male and female rats (10 of each sex per dose) were treated with a single gavage dose of etofenprox (batch Nos ST-101 and ST-102, purity 96%) at 0, 20 or 40 ml/kg bw (equivalent to 0, 21.44 and 42.88 g/kg bw, respectively). Mortality, clinical signs and body weights were recorded for 14 days. All animals were necropsied, and selected tissues (not specified) were subjected to histopathological examination.

No deaths occurred in either sex at either dose level. Treatment-related clinical signs (piloerection, hunched posture, reduced activity and reduced frequency of respiration) were observed in all rats at both levels immediately after treatment. In addition, glossy soft faeces or ochre-coloured diarrhoea was observed at 4 hours after treatment in both sexes at 20 ml/kg bw and higher, and a blood-like substance adhering to the eyelids and nostrils was observed in both sexes at 40 ml/kg bw, which persisted for up to 2 days after administration. The body weights of both sexes at 20 and 40 ml/kg bw increased throughout the observation period. Scattered diffuse haemorrhages in the lungs of all rats at both dose levels at necropsy were represented as mesh-like haemorrhage at histopathological examination. Other gross findings were liver congestion and discoloration of the kidneys, but without histopathological correlates. No oral LD_{50} value was identified; the LD_{50} is therefore greater than 40 ml/kg bw (equivalent to > 42.88 g/kg bw) in both sexes (Hashimoto, 1982a).

Table 8. Summary of the acute toxicity of etofenprox

Species/strain	Route	Sex	Parameter evaluated	Result	Reference
Rat/SD	Oral	M + F	LD_{50}	> 2000 mg/kg bw	Oda (2003a)
Rat/SD	Percutaneous	M + F	LD_{50}	> 2000 mg/kg bw	Oda (2003b)
Rat/SD	Oral	M + F	LD_{50}	> 42.88 g/kg bw[a]	Hashimoto (1982a)
	Percutaneous	M + F	LD_{50}	> 2.14 g/kg bw[a]	
	Subcutaneous	M + F	LD_{50}	> 32.16 g/kg bw[a]	
	Intraperitoneal	M + F	LD_{50}	> 42.88 g/kg bw	
Mouse/ICR	Oral	M + F	LD_{50}	> 107.2 g/kg bw[a]	Hashimoto (1982b)
	Percutaneous	M + F	LD_{50}	> 2.14 g/kg bw[a]	
	Subcutaneous	M + F	LD_{50}	> 53.6 g/kg bw[a]	
	Intraperitoneal	M + F	LD_{50}	> 53.6 g/kg bw (M) ≥ 13.4 (F)	
Rat/Wistar	Inhalation	M + F	4 h LC_{50}	> 5.88 mg/l	Jackson et al. (1983)
Dog/Beagle	Oral	1M + 1F	LD_{50}	> 5000 mg/kg bw	Harling, Burford & Heywood (1985)
Rabbit/JW	Dermal	M	Local irritancy	Non-irritant	Kashima (1985a)
Rabbit/JW	Ocular	M	Local irritancy	Non-irritant	Kashima (1985b)
Guinea-pig/Hartley	Intradermal and topical	M	Skin sensitization	Non-sensitizer	Kobayashi (1985)

F, female; LC_{50}, median lethal concentration; LD_{50}, median lethal dose; M, male

[a] A proportion of the orally, dermally and subcutaneously administered doses would not have been available for systemic absorption, and the LD_{50} values are lower than the specified values.

Dogs

Two 20-week-old Beagle dogs (one of each sex) were treated once orally, by capsule, with unformulated etofenprox (batch No. ST-103, purity 96.3%) at a dose level of 5000 mg/kg bw. Feed was offered approximately 1 hour after dosing. The dogs were closely monitored for 4 hours following treatment; thereafter, they were checked for clinical signs, body weights and feed consumption during 14 days. At the end of the observation period, a sternal puncture bone marrow smear was prepared prior to necropsy, and major organs were weighed.

The male dog showed green, semi-soft faeces within 2 hours of dose administration and semi-soft faeces of a normal colour on days 2 and 4 of the observation period. Both dogs survived the observation period. Whereas both dogs gained 700 g during the 2-week pre-dosing period, during the 2-week observation period after dosing, body weight gains were 600 and 300 g for the male and female, respectively. The female showed weight losses of up to 300 g during the 3 days following dosing and prior to necropsy. Feed consumption of both dogs was similar before and after treatment. No treatment-related effects on the bone marrow cellularity, morphology or cell distribution were observed. All organ weights were within the normal laboratory range for Beagle dogs of a similar age. The oral LD_{50} was greater than 5000 mg/kg bw in both sexes (Harling, Burford & Heywood, 1985).

(b) Dermal application

Mice

Groups of 5-week-old ICR mice (10 of each sex per dose) were treated with a single dose of etofenprox (batch No. ST-102, purity 96%) at 0, 1 or 2 ml/kg bw (equivalent to 0, 1.07 and 2.14 g/kg

bw, respectively) applied to dorsal skin. After 24 hours, the test substance was removed. Mortality, clinical signs and body weights were recorded for 14 days. All mice were necropsied, and selected tissues (not specified) were examined microscopically.

There were no deaths or treatment-related clinical signs at either dose level. Slightly reduced body weight gain occurred in males on day 1 and in females on day 3, but there were no marked changes thereafter. No treatment-related histopathology was observed at either dose level. The percutaneous LD_{50} was greater than 2 ml/kg bw (equivalent to > 2.14 g/kg bw) in both sexes (Hashimoto, 1982b).

Rats

Sprague-Dawley rats (five of each sex per dose) that were approximately 7 weeks old were treated with a single dose of etofenprox (batch No. 20024, purity 99.0%) at a dose level of 0 (dressings applied only) or 2000 mg/kg bw (1.85 ml/kg bw) by dermal application for 24 hours. The rats were observed for morbidity/mortality and clinical signs for 14 days. Body weights were recorded periodically for 14 days. All rats were necropsied after the 14-day observation period.

No deaths or treatment-related clinical signs occurred in either sex. There was no evidence of dermal irritation at the application site. No treatment-related effects on body weight gain were observed in either sex. There were no gross pathological findings at necropsy, including the application site skin, in either sex treated with etofenprox. The acute dermal LD_{50} of etofenprox in the rat was estimated to be greater than 2000 mg/kg bw in both sexes (Oda, 2003b).

Seven-week-old Sprague-Dawley-derived male and female rats (10 of each sex per dose) were percutaneously treated with a single dose of etofenprox (batch No. ST-102, purity 96%) at 0 or 2 ml/kg bw (equivalent to 0 and 2.14 g/kg bw, respectively). Mortality, clinical signs and body weights were recorded for 14 days. All animals were necropsied, and selected tissues (not specified) were subjected to histopathological examination.

No deaths occurred in either sex. Treatment-related clinical signs were transient hunched posture and reduced activity in all animals of both sexes for up to 2 hours post–dermal application. No treatment-related effects on body weight or body weight gain were observed. There were no treatment-related local or systemic gross findings at necropsy. The acute dermal LD_{50} value was greater than 2 ml/kg bw (equivalent to > 2.14 g/kg bw) (Hashimoto, 1982a).

(c) Exposure by inhalation

Rats

Three groups of five male and five female young adult Wistar rats were exposed for 4 hours by inhalation in whole-body chambers to etofenprox (batch No. ST-101, purity 96%) blended with 10% acetone as a liquid aerosol in dry air at 0 mg/l (air only), 0 mg/l (10% acetone in air) and a mean analytically determined concentration of 5.88 mg/l. Treated rats were observed for 14 days. Clinical signs, body weights and feed and water consumption were recorded throughout the study. At the end of the observation period, all animals were necropsied. The lungs were weighed and infused with fixative. The lung, liver and kidneys were examined microscopically.

The analytically determined concentrations of etofenprox in the test atmosphere at approximately hourly intervals were 5.35, 5.51, 5.65, 7.49 and 5.42 mg/l, providing a mean exposure concentration of 5.88 mg/l, with a variation in concentration of 36%. There were no deaths during the study. All animals exposed to etofenprox showed abnormal body posture during exposure, accompanied in some animals by partially or fully closed eyelids and abnormal respiratory movements. The animals exposed to acetone in air were hypoactive during exposure. Approximately 1 hour post-exposure, etofenprox-treated animals were lethargic and showed an oily appearance of the fur. No further treatment-related

clinical signs were evident within 72 hours of exposure, although some female animals showed hair loss and transient hyperactivity. A treatment-related transient weight loss was observed in males for 1 day following exposure. Thereafter, weight gain was comparable to that of the controls. A slightly reduced post-exposure weight gain of females treated with etofenprox was considered to be incidental to treatment, because the mean body weight diverged from the controls during the pre-exposure period. Females in the acetone group showed transiently reduced weight gain for 1 day following exposure.

The inhalation median lethal concentration (LC_{50}) of etofenprox at 4 hours in rats was greater than 5.88 mg/l (Jackson et al., 1983).

(d) Dermal irritation

Six adult male Japanese White rabbits were dermally exposed to 0.5 ml melted, undiluted etofenprox (batch No. ST-103, purity 96.3%), which was applied to a clipped area of dorsal skin (2.5 cm × 2.5 cm) along the midline. The applications were made once, for a 4-hour exposure duration. Dermal reactions were recorded at 30 minutes and 24, 48 and 72 hours after removal of the semi-occlusive dressing and cleaning of the test site with warm water. Animals showing dermal reactions after 72 hours were observed daily for 11 days. Five of the six rabbits showed no evidence of skin irritation at the application site throughout the observation period. The remaining rabbit showed very slight erythema at the 48- and 72-hour observation intervals. Slight erythema persisted for 7 days, after which no signs of skin irritation were apparent. No other signs of skin irritation were observed, and there were no gross findings at necropsy. Etofenprox is non-irritating to the skin of rabbits (Kashima, 1985a).

(e) Ocular irritation

In a study of primary eye irritation, 0.1 ml etofenprox (batch No. ST-103, purity 96.3%) was instilled into the right conjunctival sac of six Japanese White rabbits. The treated eyes were examined for ocular irritation reactions in the cornea, iris and conjunctivae 1, 24, 48 and 72 hours after instillation. All eyes showed minimal (grade 1) erythema 1 hour after instillation, accompanied in one animal by minimal (grade 1) oedema. Five animals at 24 hours and three animals at 48 hours continued to show grade 1 erythema, but without oedema. There were no corneal or irritable reactions in any animal at any examination, and all animals were free of ocular reactions at 72 hours. Under the conditions of this study, it was concluded that etofenprox is non-irritating to the eyes of rabbits (Kashima, 1985b).

(f) Dermal sensitization

In a study of dermal sensitization of etofenprox, intradermal and topical induction doses of etofenprox (batch No. ST-103, purity not specified), dinitrochlorobenzene or the vehicle only (corn oil) were applied to 20 male guinea-pigs (English Hartley strain). The skin reaction scores for all vehicle control and etofenprox-treated groups were zero (no reaction) at the 24-, 48- and 72-hour observation periods. Etofenprox is not a dermal sensitizer in the guinea-pig maximization test (Kobayashi, 1985).

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

Five groups of 20 male and 20 female 5- to 6-week-old Swiss mice (CD-1 strain) were administered etofenprox (batch No. ST-101, purity 96%) without correction for purity in the diet (with corn oil) at a concentration of 0, 50, 500, 3000 or 15 000 ppm (equal to 0, 6.1, 60, 375 and 1975 mg/kg bw per day in males and 0, 6.9, 71, 390 and 2192 mg/kg bw per day in females, respectively) for 13 weeks. The concentrations, stability and homogeneity of the diets were analysed. Morbidity/mortality and clinical signs were checked once or twice daily, and body weights and water consumption were recorded throughout the study. Ophthalmology, haematology, blood chemistry and

urine analyses were performed. Blood samples from the orbital sinus were withdrawn from non-feed-deprived mice. Water and feed were removed overnight from animals to be sampled for urinalysis. All surviving animals were necropsied after 13 weeks of treatment. The animals found dead or moribund were also necropsied. After necropsy, selected organs were weighed, and samples of major organs/tissues and gross lesions except aorta, caecum, mammary gland/skin, sciatic nerve, seminal vesicles and tongue were examined histopathologically. Two additional sections were stained with periodic acid Schiff or Oil Red O.

The concentrations, stability and homogeneity of the diets were acceptable. Treatment-related deaths occurred at 15 000 ppm. Two males and six females died or were killed in extremis. One female at week 6 and one male at week 9 were found dead with cannibalism and advanced autolysis, which precluded necropsy. In addition, one male and five females were euthanized at week 13 because of their deteriorating conditions (piloerection, hunched posture, emaciated appearance, anaemic appearance, lethargy, body tremors, unsteady gait or respiratory distress). These animals revealed kidney damage, minimal adipose tissue and small thymus in all the females and kidney enlargement in the male at necropsy. Treatment-related clinical signs were confined to high-dose animals only and were more severe in the females. A male at 50 ppm and another at 3000 ppm died after the final blood sampling procedure without any clinical signs; their lungs were congested at necropsy, but their deaths are not considered to be related to the treatment. Most of the clinical signs at 15 000 ppm were first apparent during the first 6 weeks of treatment, but tremors and unsteady gait appeared at week 12 or 13. The overall male and female feed consumption at 15 000 ppm was significantly lower than that in controls. The overall male and female body weight gains at 15 000 ppm were markedly depressed by 69.7% and 82.8%, respectively. Water consumption at 15 000 ppm was consistently increased in both sexes. The treatment-related changes in haematology, blood biochemistry, organ weights and histopathological changes are summarized in Table 9.

Treatment-related anaemia (slight decreases in haematocrit, haemoglobin concentration and red blood cell counts) was evident in both sexes at 15 000 ppm at weeks 6 and 13. Males at 3000 ppm showed decreases in haemoglobin concentration and red blood cell counts at week 13; however, these changes in haematology in males at 3000 ppm were not considered toxicologically relevant. Although males at 50 or 500 ppm showed significant decreases in red blood cell counts and haemoglobin at week 6, these effects are not considered to be treatment related because the values showed no dose dependency. Significant elevation of white blood cell counts, due to increased numbers of neutrophils and lymphocytes, was also apparent in both sexes at 15 000 ppm. At 15 000 ppm, elevated blood urea nitrogen and cholesterol were observed in females at weeks 5 and 12 and in males at week 5. Although blood urea nitrogen concentrations of females at 50, 500 and 3000 ppm were also significantly higher than control values at week 5, these changes were not associated with renal morphological alterations or changes in the kidney weights at doses up to 3000 ppm and altered urinalysis indicative of renal damage. Therefore, increases in blood urea nitrogen at doses up to 3000 ppm were not considered to be toxicologically relevant. The glucose level was slightly decreased in both sexes at 15 000 ppm at weeks 5 and 13 and in females at 3000 ppm at week 13. The decreases at 15 000 ppm were due to lower body weights. Although several other minor, but statistically significant, differences from the control values were observed in the blood chemistry profile, they were not considered to be toxicologically significant. The urinalysis indicated that specific gravity and pH values were low in males at 15 000 ppm compared with controls.

Surviving mice at termination and five females necropsied before termination at 15 000 ppm revealed cortical scarring in the kidney, small thymus and minimal adipose tissue. Increased liver and kidney weights were observed in both sexes at 15 000 ppm. No other organ weights were affected at any dose level. Treatment-related histopathological alterations were widespread tubular basophilia, extensive, frequently cystic tubular dilatation and renal pelvis dilatation in the kidney, minimal to slight centrilobular hepatocyte enlargement without fat storage and glycogen accumulation, increased

Table 9. Summary of treatment-related changes in a 90-day repeated-dose toxicity study in mice

Parameters	Males					Females				
	Dietary concentration (ppm)									
	0	50	500	3000	15 000	0	50	500	3000	15 000
Haematology										
Week 6										
Packed cell volume (%)	46	43	44	44	44	44	45	45	45	42*
Haemoglobin (g/dl)	14	13.2*	13.2*	12.8*	13.1**	13.8	13.7	14.1	14	13
Red blood cells ($\times 10^6$/mm^3)	6.6	6.1**	5.9**	5.9**	6.2**	6.4	6.3	7	6.7	6.1
White blood cells ($\times 10^3$/mm^3)	3.5	4.3	3.7	3.9	5.5**	3.2	4.9	4.2	4.3	6.3**
Week 13										
Packed cell volume (%)	47	47	46	44*	43**	44	44	46	43	39**
Haemoglobin (g/dl)	14.1	14	14.4	13.3*	12.6**	14	14	15	14	12.4*
Red blood cells ($\times 10^6$/mm^3)	7.7	7.5	7.6	7.2*	7.0**	7.5	7.6	7.8	7.4	6.8**
White blood cells ($\times 10^3$/mm^3)	5.9	4.7	6.9	4.8	5.3	3.1	4.9	4.5	3.4	5.1**
Selected clinical chemistry values										
Week 5										
Glucose (mg/dl)	179	189	177	189	152*	183	178	176	166	136**
Urea nitrogen (mg/dl)	20	19	21	17	24*	18	24*	22*	27**	38**
Cholesterol (mg/dl)	120	106	106	97	142*	91	75	76	80	128**
Week 12										
Glucose (mg/dl)	185	196	183	228	140*	174	169	174	147**	118**
Urea nitrogen (mg/dl)	25	25	24	30	23	19	25	26	21	35**
Cholesterol (mg/dl)	105	102	109	79	110	67	65	75	66	117**
Absolute and relative organ weights										
Liver weight (g)	2.348	2.354	2.307	2.329	2.065*	1.748	1.682	1.674	1.83	1.606
Liver weight (%)	6.12	5.85	5.89	6.12	7.02**	6.05	5.72	5.84	6.26	7.04***
Kidney weight (g)	0.675	0.714	0.663	0.654	0.753*	0.463	0.433	0.461	0.428	0.577***
Kidney weight (%)	1.77	1.77	1.69	1.72	2.72**	1.61	1.49	1.61	1.47	2.90***
Incidence of selected histopathological alterations										
No. of animals examined	20	19	20	19	18	20	20	20	20	19
Kidneys										
Focal tubular basophilia	1	0	0	1	2	0	0	0	1	1
Widespread tubular basophilia	0	0	0	0	16	0	0	0	0	16
Extensive tubular dilatation	0	0	0	0	14	0	0	0	0	16
Renal pelvic dilatation	0	0	1	0	5	0	0	0	0	5
Liver										
Hepatocyte enlargement	3	2	1	2	12	0	0	0	0	10
Lymphoreticular system										
Increased cellularity of splenic white pulp	3	3	0	1	9	2	2	0	2	7
Lymphoid hyperplasia, lymph node	2	1	5	7	8	1	1	1	1	5
Reduced thymic cellularity	0	0	0	0	4	0	0	1	0	2

From Green et al. (1983b)
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

cellularity of the splenic white pulp (especially T-cell zone), lymphoid hyperplasia in the lymph nodes and reduced thymic cellularity at 15 000 ppm. The reactive change in the lymph nodes in males at 500 and 3000 ppm was minor and not significant.

The no-observed-adverse-effect level (NOAEL) was 3000 ppm (equal to 375 mg/kg bw per day), based on increased mortality and the occurrence of reduced body weight gain and feed consumption, increased water consumption, minor haematological effects, histopathological alterations indicative of kidney damage and minor changes in the liver and the lymphoreticular system at 15 000 ppm (equal to 1975 mg/kg bw per day) (Green et al., 1983b).

Rats

A 90-day dietary toxicity study in rats was conducted with groups of 5- to 6-week-old Sprague-Dawley rats (20 of each sex per dose) administered diets containing etofenprox (batch No. ST-101, purity 96%) at a concentration of 0, 50, 300, 1800 or 10 800 ppm without correction for purity (equal to 0, 3.3, 20, 120 and 734 mg/kg bw per day in males and 0, 3.8, 23, 142 and 820 mg/kg bw per day in females, respectively). The concentrations, stability and homogeneity of the diets were analysed. Mortality and clinical signs were checked once or twice daily, and body weights and feed and water consumption were measured throughout the study. Ophthalmoscopy, haematology, blood chemistry, including thyroid function measurements, and urinalysis were performed. All rats were necropsied, and macroscopic examination was performed. Major organs and tissues were weighed and examined microscopically.

The concentrations, stability and homogeneity of the diets were acceptable. One control male died accidentally at week 13, probably due to an orbital blood sampling error. No treatment-related clinical signs were observed in either sex at any dose level. The overall body weight gains were decreased by 15.8% and 10.1% in males and females, respectively, at 10 800 ppm. A similar but not significant decrease (8.0%) was detected in females at 1800 ppm. Feed consumption was not affected by the treatment at any dose level in males, and a slight but significant decrease in feed consumption was observed in females at 10 800 ppm. Water consumption was reduced by 10.8–18.4% in males and by 6.9–11.1% in females at 10 800 ppm. There were no treatment-related ocular lesions. The treatment-related changes in haematology, blood biochemistry, organ weights and histopathological changes are summarized in Table 10.

There were no treatment-related effects on haematology except for increased clotting indices in males at 10 800 ppm at weeks 6 and 12. Further investigations at weeks 13 and 14 also demonstrated significant prolongation of the prothrombin time and activated partial thromboplastin time in males at the same dose, suggesting effects of etofenprox on the intrinsic and extrinsic systems. Hepatotoxicity described below might be related to the effects on the clotting systems. Slight but statistically significant increases in alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activities (no statistical significance) in males at 1800 and 10 800 ppm were detected at week 6. Cholesterol concentrations were significantly elevated in males at 10 800 ppm at weeks 6 and 12 and in males at 1800 ppm and females at 10 800 ppm at week 12 only. These changes in biochemical parameters indicated adverse effects on the liver. Plasma concentrations of thyroxine (T_4) were reduced in males, but not in females, at 1800 and 10 800 ppm. A slight increase in triiodothyronine (T_3) was detected in females at 10 800 ppm at week 6 only. Thyroid stimulating hormone (TSH) levels were not measured in this study. No treatment-related changes were observed in urinalysis in either sex at any dose.

The adjusted (to body weights) liver and adrenal weights of both sexes at 10 800 ppm were significantly increased. Females at 1800 ppm showed a similar but less marked effect on the liver. The absolute and adjusted thyroid weights in males at 1800 and 10 800 ppm were increased, although statistical significance was achieved for adjusted thyroid weights only. The absolute and adjusted thyroid weights of all treated female groups and the male groups at 50 and 300 ppm were

Table 10. Summary of treatment-related changes in a 90-day repeated-dose toxicity study in rats

Parameters	Males					Females				
	Dietary concentration (ppm)									
	0	50	300	1800	10 800	0	50	300	1800	10 800
Group mean blood clotting indices										
Week 12										
TT (s)	26.2	26.2	26.7	27.1	39.2**	24.6	24.1	23.1	24.9	22.8*
Week 13										
PT (s)	11.5	—	—	—	11.8	—	—	—	—	—
APTT (s)	18.7	—	—	—	25.5***	—	—	—	—	—
Week 14										
TT (s)	25.8	—	—	—	33.9***	—	—	—	—	—
PT (s)	11.4	—	—	—	13.4***	—	—	—	—	—
APTT (s)	20.7	—	—	—	35.3***	—	—	—	—	—
Clinical chemistry values										
Week 6										
ALT (mU/ml)	27	28	29	35*	42**	20	19	24	22	20
AST (mU/ml)	56	64	66	74**	84**	56	54	55	52	55
LDH (mU/ml)	598	804	872	1036	1675**	584	468	505	498	471
Chol (mg/dl)	49	51	51	51	60**	51	58	53	53	56
T_3 (ng/dl)	42	49	45	45	45	47	48	47	48	60*
T_4 (mg/dl)	7.2	7.3	7.8	6.3	5.7**	5.3	4.8	6.1	5.6	6.1
Week 12										
ALT (mU/ml)	29	23	34	31	34*	23	17*	21*	18*	17**
AST (mU/ml)	59	63	73	55	53	51	49	52	50	53
LDH (mU/ml)	262	417	379	233	282	188	239	231	221	268
Chol (mg/dl)	37	39	41	44*	52**	39	49	44	42	58**
T_3 (ng/dl)	46	52	55	51	51	53	51	54	57	56
T_4 (mg/dl)	5.2	4.8	4.9	4.3*	3.9**	3.0	2.8	2.8	3.0	3.0
Selected absolute and relative organ weights										
Liver weight (g)										
- absolute	22.8	23.4	22.7	24.3	27	12.2	11.7	12.4	12.8	15.7
- adjusted[a]	22.2	22.8	23	23.4	28.8**	11.9	11.7	12.2	13.0*	16.1*
Thyroid weight (mg)										
- absolute	23	24	24	27	28	21	21	20	22	24
- adjusted[a]	22	24	24	27*	29**	21	21	20	22	24
Adrenal weight (mg)										
- absolute	58	55	59	59	65	77	79	74	82	87
- adjusted[a]	57	54	60	57	67**	76	79	73	82	88**
Treatment-related histopathological findings										
Incidence of hepatocyte enlargement	0/19	0/20	0/20	0/20	0/20	0/20	0/20	0/20	1/20	9/20
Incidence of thyroid microfollicles	10/19	11/20	5/20	19/20	18/20	0/20	0/20	0/20	2/20	9/20

From Green et al. (1983a)

APTT, activated partial thromboplastin time; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Chol, cholesterol; LDH, lactate dehydrogenase; PT, prothrombin time; T_3, triiodothyronine; T_4, thyroxine; TT, thrombotest; U, units; —, not investigated;
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

[a] Statistically adjusted using body weight as covariate.

not significantly different from the controls. There were no other treatment-related effects on organ weights at any doses.

Treatment-related histopathological findings were detected in the liver and thyroid. Minimal centrilobular enlargement of hepatocytes occurred in females at 10 800 ppm. The enlargement was not evident in males at any dose level, despite the increase in the adjusted liver weights of males at 10 800 ppm. Oil Red O and periodic acid Schiff staining of liver sections revealed no treatment-related alterations, indicating no increase in glycogen or fat in the hepatocytes. Minimal to moderate numbers of microfollicles in the thyroid were increased in both sexes treated at 10 800 ppm. The treatment-related histopathological findings in thyroid were not correlated to the thyroid weights in females. No other histopathological alterations were considered to be treatment related.

The NOAEL was 300 ppm (equal to 20 mg/kg bw per day), based on the liver toxicity (hepatocyte enlargement and clinical evidence of liver dysfunction affecting fat metabolism and the synthesis of blood clotting factors) and thyroid toxicity (an increase in the number of thyroid microfollicles and reduced levels of circulating T_4) at 1800 ppm (equal to 120 mg/kg bw per day) (Green et al., 1983a).

Dogs

Four groups of four male and four female Beagle dogs (22–27 weeks old) were treated orally with etofenprox (batch No. ST-103, purity 96.3%) in the diet (in corn oil) at 0, 100, 1000 or 10 000 ppm without correction for purity (equal to 0, 3.46, 33.4 and 352 mg/kg bw per day in males and 0, 3.17, 32.2 and 339 mg/kg bw per day in females, respectively) for at least 52 weeks. A recovery study was conducted using two further groups in which two dogs of each sex per group were treated with etofenprox at 0 or 10 000 ppm for 52 weeks and observed for 8 weeks after the cessation of the treatment. The concentrations, homogeneity and stability of the diets were analysed. The dogs were frequently checked for clinical signs of toxicity. Body weights and feed consumption were recorded throughout the study. All dogs were examined for ocular changes before exposure and during treatment weeks 6, 12, 25 and 51, using an indirect ophthalmoscope. Haematology, serum clinical chemistry, including ornithine carbamoyltransferase activity, and urinalysis were performed on all dogs (feed-deprived) before and during the treatment and at the end of the 8-week recovery period. All main group dogs were necropsied after at least 52 weeks of treatment, and recovery group animals were necropsied 8 weeks after the cessation of the treatment period. Macroscopic examination was performed on all animals. Selected organs were weighed, and all major organs/tissues were examined microscopically. Additional sections of liver and kidneys were stained with Oil Red O to confirm the presence of fat.

The homogeneity, stability and concentrations of etofenprox in the diets were within the acceptable range. No deaths or treatment-related clinical signs were observed. Feed consumption and body weight gain throughout the study were unaffected by the treatment. There were no treatment-related ophthalmological findings. Red blood cell counts and haemoglobin levels were slightly decreased at weeks 25 and 52 in males at 10 000 ppm, indicating slight anaemia. These decreases in red blood cells and haemoglobin values were not considered to be treatment related because the changes were minor and of doubtful toxicological significance. A lower level of haemoglobin in males at 10 000 ppm at week 6 was not considered to be adverse because of the lower value at pretest in the same group. All changes in haematological parameters in the etofenprox-treated groups were not considered to be toxicologically significant because the changes were small, sometimes within normal range and in some cases did not achieve statistical significance.

Total protein and serum albumin concentrations were slightly but significantly decreased from week 6 of the treatment in both males and females at 10 000 ppm (Table 11). Serum cholesterol concentrations were also decreased in these animals. These changes in total protein, serum albumin and cholesterol levels were statistically significant at all intervals except in females at week 51. Serum alkaline phosphatase activities in both sexes at 10 000 ppm were increased at all sampling intervals

Table 11. Summary of blood biochemistry parameters of 1-year toxicity study in dogs

Parameter / time point (weeks)	Males				Females			
	Dietary concentration (ppm)							
	0	100	1000	10 000	0	100	1000	10 000
Protein (g/dl)								
Pre-dose	5.5	5.6	5.8	5.5	5.4	5.5	5.2	5.4
6	5.7	5.8	5.9	5.2*	5.7	6.0*	5.7	4.8**
12	5.7	5.6	5.3	5.2*	5.5	5.8	5.5	5.0*
25	6.0	6.0	5.8	5.2**	6.1	6.2	5.8	5.4**
51	6.2	6.0	6.2	5.3**	6.0	6.1	5.9	5.6**
60 (recovery)	6.7	—	—	6.4	6.6	—	—	6.2
Albumin (g/dl)								
Pre-dose	3.0	3.2	3.0	3.0	3.1	3.2	3.1	3.1
6	3.3	3.4	3.3	2.9**	3.5	3.4	3.5	2.8**
12	3.1	3.1	2.9	2.6**	3.1	3.1	3.1	2.6**
25	3.2	3.2	3.0	2.6**	3.2	3.3	3.2	2.7**
51	3.2	3.0*	3.0*	2.6**	3.2	3.2	3.1	2.7**
60 (recovery)	3.5	—	—	3.3	3.4	—	—	3.4
Serum alkaline phosphatase (mU/ml)								
Pre-dose	248	228	196	231	258	267	284	239
6	213	182	199	279	208	212	246	267*
12	201	173	194	334**	219	196	241	249
25	147	129	143	345**	151	110	160	217
51	117	108	173	322*	132	90	179	264*
60 (recovery)	188	—	—	151	153	—	—	111**
Cholesterol (mg/dl)								
Pre-dose	162	148	154	129*	140	149	147	142
6	171	157	159	121**	152	143	160	112**
12	151	146	140	107**	139	141	142	109*
25	140	119	123	96**	162	126	150	111*
51	139	117	139	100**	149	125	168	140
60 (recovery)	160	—	—	132	202	—	—	117

From Harling et al. (1985)
U, units; * $P < 0.05$; ** $P < 0.01$

from week 6, with statistical significance achieved in males at weeks 12, 25 and 51 and in females in weeks 6 and 51. These changes in serum clinical chemistry parameters returned to the normal range at the end of the recovery period. There were no treatment-related effects on the cellular or chemical constituents of urine at any dose level.

No treatment-related gross findings were detected at necropsy. The absolute and relative (to body weights) liver weights were increased in both sexes at 10 000 ppm after 52 weeks of treatment, and increased tendencies were detected after the recovery period. The increases in absolute but not relative weights of the kidney in females and of lungs and pancreas in both sexes at 10 000 ppm were not considered to be treatment related because there were no biochemical or histopathological alterations in these organs. Swelling of centrilobular hepatocytes was observed in two females at

10 000 ppm after 52 weeks of treatment. After the recovery period, this change in the liver was not detected in females treated at 10 000 ppm.

The NOAEL was 1000 ppm (equal to 32.2 mg/kg bw per day), based on effects on hepatotoxicity, including increased liver weights in both sexes and histopathological alterations in females at 10 000 ppm (equal to 339 mg/kg bw per day). The hepatic effects were reversible (Harling et al., 1985).

(b) Dermal application

Rabbits

Four groups of 10 male and 10 female 12- to 13-week-old New Zealand White rabbits were treated with technical etofenprox (batch No. 21049, purity 99.18%) by dermal application daily for 28 days at a dose level of 0 (water only), 400, 650 or 1000 mg/kg bw per day. Two additional groups of 10 rabbits of each sex were similarly treated for 28 days at 0 or 1000 mg/kg bw per day and maintained untreated for 14 days after the cessation of dosing to examine recovery. Dermal applications were made for 6 hours/day onto a shaved, non-abraded area of dorsal skin (approximately 10% of the body surface area) under semi-occlusive dressings. After 6 hours of contact, the dressings were removed, and any remaining test substance was removed from the application sites by wiping with dilute soap solution, followed by water only. The rabbits were examined for mortality and clinical signs daily. The skin application sites were examined for signs of erythema, oedema or other skin reactions. Body weights and feed consumption were recorded pre-dosing and throughout the study. All animals were given an ophthalmoscopic examination pre-dosing and towards the end of the treatment and recovery periods. Haematology and clinical chemistry determinations were performed on blood samples withdrawn from feed-deprived animals. After 28 days of treatment or following the 14-day recovery period, all rabbits were necropsied and examined macroscopically. Selected organs were removed and weighed. All tissues from the animals at 0 or 1000 mg/kg bw per day (including the recovery group) and application site skin and gross lesions from all study rabbits were examined microscopically.

There were no deaths or treatment-related clinical signs at any dose level. The incidence of erythema at the application site skin in the treated groups was slightly higher than in the controls throughout the study. Scabbing, desquamation or exfoliation, oedema, fissuring and thickening of the application sites also tended to be slightly more prevalent in the treated group. There were no treatment-related changes in the body weights, body weight gains, feed intake, ocular findings, or haematological and clinical chemistry parameters. A marginally shorter prothrombin time in the 1000 mg/kg bw per day group males and a minimally lower proportion of lymphocytes in the 1000 mg/kg bw per day group males were not considered to be toxicologically significant. There were no treatment-related gross necropsy findings, organ weight changes or systemic histopathological changes. Treatment-related histopathological alterations in the application sites were focal epidermal hyperplasia, chronic dermal infiltration and dermal heterophil infiltrates in males and diffuse epidermal hyperplasia in females at all dose levels. Recovery group animals at 1000 mg/kg bw per day showed evidence of resolution of the skin lesions; however, the incidences of focal epidermal hyperplasia and heterophil infiltration in males and focal chronic inflammation in females remained slightly raised.

The NOAEL for systemic toxicity was 1000 mg/kg bw per day, based on the absence of systemic effects at this dose level, the highest dose tested (Killeen, 2000).

(c) Exposure by inhalation

Rats

A 13-week inhalation toxicity study was conducted in rats. Five groups of 15 male and 15 female 6- to 7-week-old Wistar rats were exposed to an aerosol produced from a 9:1 weight per weight (w/w) solution of etofenprox (batch No. ST-103, purity 96%) in acetone at a concentration

of 0 (air only), 0 (vehicle), 0.04, 0.20 or 1.0 mg/l (equivalent to mean analytically determined concentrations of 0 [air], 0 [vehicle], 0.042, 0.21 and 1.01 mg/l, respectively) in whole-body exposure chambers for 6 hours/day, 6 days/week, for 13 weeks. In a range-finding study, minor superficial scab formation and transient weight loss were observed at an apparent concentration of 4.77 mg/l, but not at 1.19 mg/l. The high dose level of 1.0 mg/l was selected for the main study based on technical limitations of atmosphere generation due to compound deposition in the aerosol delivery system. The actual exposure concentrations, the homogeneity of the test article, distribution within the exposure chambers and the particle size distribution of the atmospheres were measured. Mortality and clinical signs were checked daily. Body weights, feed consumption and water consumption were recorded throughout the study. Blood samples were collected from overnight-fasted rats for haematology and plasma clinical chemistry parameters, including T_3 and T_4 measurements, which were performed at weeks 5 and 12. TSH levels were not evaluated in this study. All rats were necropsied, and selected organs were weighed. All tissues from the animals treated at 0 (air) or 1.01 mg/l and major tissues and gross lesions from all animals of all groups were examined microscopically.

The analytically determined atmospheric concentrations of etofenprox were in good agreement with the target concentrations. The multiple homogeneity analyses indicated a homogeneous distribution of etofenprox within the chambers. Particle size distribution analysis indicated that a large proportion of particles at all concentrations were respirable (less than 5.5 µm). There were no deaths during the study. Scab formation on the skin of the back of the ears was observed in both sexes at 1.01 mg/l and in females at 0.21 mg/l during the early part of the exposure. Scab formation was considered to result from compound deposition on the fur, resulting in excessive grooming overnight. The effect was accompanied by local hair loss at 1.01 mg/l. There were no treatment-related effects on body weight gain in either sex at any dose level. The overall mean feed consumption in females at 1.01 mg/l was slightly greater, but the effect is considered not to be of toxicological significance. Slight increases in the overall mean water consumption of all etofenprox-treated groups were judged not to be adverse because of the absence of histopathological effects in the kidneys.

No ocular abnormalities were detected. Slight decreases in red blood cell counts at weeks 5 and 12, haemoglobin at week 5 and mean corpuscular haemoglobin concentration at week 5 in males at 1.01 mg/l, considered to be indicative of slight anaemia, were observed. There were statistically significant differences between treated and control groups at week 5 and/or week 12 for a number of haematological parameters. The alterations in haematological parameters were considered not to have toxicological relevance because the changes were minor and were within the normal range for Wistar rats. Etofenprox treatment had no effect on blood T_3 or T_4 levels. There were statistically significant differences in clinical chemistry parameters between the treated and control groups at week 5 and/or week 12 for glucose, total protein, albumin, creatinine, albumin to globulin ratio, AST and ALT activities, chloride and cholesterol. These alterations in clinical chemistry parameters were not judged to be toxicologically relevant, as the differences were minor.

There were no treatment-related macroscopic findings. Treatment-related increases in the weights of the thyroid (both sexes at 1.01 mg/l), liver (both sexes at 1.01 mg/l, females at 0.21 mg/l) and kidneys (both sexes at 1.01 and 0.21 mg/l) were observed. No histopathological changes in kidney weights and no biochemical changes indicating renal toxicity were observed. An increase in adrenal weights was also detected in females at 1.01 mg/l. Although the weights of the heart and lungs in males at 1.01 and 0.21 mg/l and ovary weights at 1.01 mg/l were higher than those of the control groups, there were no correlating histopathological findings. Therefore, the differences in weights were considered to be incidental to treatment.

Treatment-related histopathological alterations were enlargement of centrilobular hepatocytes in both sexes at 1.01 mg/l and increased numbers of microfollicles and increased height of the follicular epithelium in the thyroid in males at 1.01 mg/l. Slightly increased adrenal cortical widths in females at 0.21 and 1.01 mg/l (3/15 and 4/15, respectively) compared with the controls (1/15)

were not considered to be adverse effects of the treatment. All other histopathological findings were considered to be unrelated to treatment.

The no-observed-adverse-effect concentration (NOAEC) was 0.21 mg/l, based on the occurrence of increased liver and thyroid weights (in both sexes) and histopathological alterations in the thyroid in males at 1.01 mg/l (Coombs et al., 1985).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Groups of 52 male and 52 female 6-week-old Swiss mice were treated orally with etofenprox (batch No. ST-103, purity 96.3%) in the diet (in corn oil) for at least 104 weeks at 0, 30, 100, 700 or 4900 ppm without correction for purity (equal to 0, 3.1, 10.4, 75.2 and 546.9 mg/kg bw per day in males and 0, 3.6, 11.7, 80.9 and 615.5 mg/kg bw per day in females, respectively). Subgroups (24 animals of each sex per group) were treated in parallel with the main groups; 10 animals of each sex per group were killed after 26 weeks of treatment, and the remaining animals were killed after 52 weeks of treatment. The dose levels were selected on the basis of a 13-week toxicity study (Green et al., 1983b). The stability and homogeneity of the diet formulations were analysed prior to the start of the study. The concentrations were measured at week 1 and subsequently at 3-month intervals. Animals were observed for mortality, clinical signs of toxicity or palpable masses. Animals found dead and those killed in extremis were necropsied. Body weights, feed consumption and water consumption were recorded throughout the study. Ophthalmic examinations were performed on all animals at 0 or 4900 ppm. Blood and urine samples were collected for haematology, clinical chemistry and urinalysis at weeks 14–16, 24–26, 50/52, 76–78 and 101–103. Survivors from the main study groups were necropsied, and organ weights were recorded after at least 104 weeks of treatment. Blood smears were prepared from all animals killed during the study. Tissue/organ samples, including all gross lesions suggestive of neoplasia, were preserved from all decedents and all survivors killed after 26, 52 and 104 weeks and at termination and subjected to histopathological evaluation. Additional sections of liver and kidney were stained with Oil Red O and periodic acid Schiff.

The homogeneity, stability and achieved concentrations of etofenprox in the diets were acceptable. There were no treatment-related clinical signs of toxicity at any dose level in either sex, other than slight pallor of the extremities in a few male mice at 4900 ppm. Survival incidences at termination were 46%, 44%, 27%, 35% and 19% in males and 54%, 54%, 48%, 44% and 54% in females at 0, 30, 100, 700 and 4900 ppm, respectively. The body weight gains of both sexes at 4900 ppm were significantly reduced during the first 52 weeks of treatment, leading to reductions in overall weight gain of 27.8% and 13.8% in males and females, respectively. Males at 700 ppm showed a significant decrease in overall weight gain at weeks 78–107. There was no effect of treatment on feed consumption in either sex at any dose level. Water consumption was significantly increased by up to 51.7% at 4900 ppm during weeks 12 and 23 in males and at weeks 5, 12 and 23 in females. Males at 700 ppm showed significantly higher water consumption at week 23.

There were no treatment-related ophthalmological findings in either sex. Dose-dependent changes indicating slight anaemia were observed in haematological parameters during the first 52 weeks of the study in males at 100 ppm and higher (Table 12). Other treatment-related effects on haematological parameters were increased platelet counts in females at 4900 ppm at weeks 78 and 101. There were no effects on the clinical chemistry parameters. Treatment-related effects on urinary parameters were a larger volume of more dilute urine in males at week 52 and reduced urinary specific gravity in males at weeks 77 and 102 at 4900 ppm.

Treatment-related macroscopic findings at necropsy included cortical scarring of the kidney, which occurred in females at 4900 ppm (Table 13). Pallor of the kidneys occurred at a higher incidence in both sexes at 100, 700 or 4900 ppm, and masses or unilateral enlargement in males at 4900 ppm,

Table 12. Summary of alterations in haematological parameters and organ weights in a carcinogenicity study in mice with etofenprox

Parameter	Group mean haematological parameters / organ weights									
	Males					Females				
	Dietary concentration (ppm)									
	0	30	100	700	4900	0	30	100	700	4900
Haematological parameters										
Week 15										
Packed cell volume (%)	48	49	48	48	47	47	47	49	48	46
Haemoglobin (g/dl)	14	14.2	14	13.6	12.9**	13.8	13.9	14.1	13.9	13.1*
Red blood cells (10^6/mm^3)	7.5	7.5	7.3	7.1	7.0*	7	7.2	7.4	7.3	6.6
MCHC (%)	29.4	29.1	28.9	28.3*	27.8**	29.4	29.7	28.9	28.9	28.7
MCV (fl)	64	65	66	68*	66*	67	65	66	66	69
Week 26										
Packed cell volume (%)	45	44	46	47	44	46	47	47	47	44
Haemoglobin (g/dl)	14.4	14.1	13.7	14.3	12.8**	13.8	14.3	14.5	14.5	13.4
Red blood cells (10^6/mm^3)	7.6	7.2	7	7.5	6.8*	7.2	7.3	7.5	7.1	6.5
MCHC (%)	31.7	32.1	29.8*	30.2*	28.9**	30	30.5	30.8	30.6	30.3
MCV (fl)	60	61	67*	63*	65**	65	65	63	67	68
Week 52										
Packed cell volume (%)	46	43	50	46	42*	45	45	46	48	46
Haemoglobin (g/dl)	15.3	14.3	15.5	13.5	12.7**	14.1	14.4	14.6	15.3	14.5
Red blood cells (10^6/mm^3)	7.8	7.2	8	6.6	6.3**	6.9	7	7.4	7.9	7.2
MCHC (%)	33.5	33.3	31.0*	29.2**	30.3**	31.9	32.1	31.8	31.5	31.9
MCV (fl)	59	61	63	74**	68**	65	65	63	62	64
Week 101										
Packed cell volume (%)	45	44	43	48	43	41	40	41	39	40
Haemoglobin (g/dl)	13	13.1	12.8	13.5	12.4	12	12.1	12.2	11.4	11.4
Red blood cells (10^6/mm^3)	7.6	7.6	7.2	7.9	6.7	6.7	6.9	6.6	6.4	6.2
MCHC (%)	29	30	29.5	28.3	28.4	28.6	30	29.4	28.9	28.5*
MCV (fl)	59	58	60	61	66**	67	59	65	62	65*
Organ weights										
Week 26										
Liver[a] (g)	2.09	2.02	2.17	2.11	2.43*	1.55	1.59	1.69	1.72	1.63
Spleen (g)	0.132	0.114	0.103	0.142	0.141	0.121	0.12	0.119	0.116	0.122
Week 52										
Liver[a] (g)	2.27	2.35	2.48	2.4	2.35	2.09	1.93	1.81	1.81	1.85
Spleen (g)	0.121	0.134	0.123	0.135	0.160*	0.158	0.13	0.108	0.111	0.141
Termination										
Liver[a] (g)	2.87	2.83	2.94	2.8	3.16	1.83	1.9	1.99	1.87	2.01**
Spleen (g)	0.138	0.169	0.127	0.117	0.105	0.157	0.223	0.239	0.232	0.19

From Green et al. (1986b)

MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; * $P < 0.05$; ** $P < 0.01$

[a] Weights adjusted using body weight as covariate before statistical analysis.

Table 13. Summary of non-neoplastic histopathological findings in kidney in carcinogenicity study in mice with etofenprox

	Incidence of finding									
	Males					Females				
	Dietary concentration (ppm)									
	0	30	100	700	4900	0	30	100	700	4900
Week 26										
No. examined	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*
Dilated/basophilic tubules[a]	0	0	0	0	2	1	0	0	0	1
- grade[b]	[0, 0, 0, 0, 0]	[0, 0, 0, 0, 0]	[0, 0, 0, 0, 0]	[0, 0, 0, 0, 0]	[0, 2, 0, 0, 0]	[1, 0, 0, 0, 0]	[0, 0, 0, 0, 0]	[0, 0, 0, 0, 0]	[0, 0, 0, 0, 0]	[0, 1, 0, 0, 0]
Week 52										
No. examined	*13*	*8*	*13*	*13*	*12*	*12*	*13*	*14*	*13*	*13*
Dilated/basophilic tubules[a]	0	0	0	0	5	1	0	0	3	4
- grade[b]	[0, 0, 0, 0, 0]	[0, 0, 0, 0, 0]	[0, 0, 0, 0, 0]	[0, 0, 0, 0, 0]	[2, 0, 2, 1, 0]	[1, 0, 0, 0, 0]	[0, 0, 0, 0, 0]	[0, 0, 0, 0, 0]	[3, 0, 0, 0, 0]	[3, 0, 1, 0, 0]
Termination										
No. examined	*24*	*23*	*14*	*18*	*10*	*28*	*28*	*25*	*23*	*28*
Dilated/basophilic tubules[a]	5	4	5	8	6	3	0	2	4	15
- grade[b]	[5, 0, 0, 0, 0]	[3, 1, 0, 0, 0]	[3, 2, 0, 0, 0]	[6, 2, 0, 0, 0]	[3, 0, 3, 0, 0]	[3, 0, 0, 0, 0]	[0, 0, 0, 0, 0]	[2, 0, 0, 0, 0]	[3, 1, 0, 0, 0]	[6, 4, 5, 0, 0]
Dilated/cystic Bowman's capsule	1	3	2	8	2	2	2	3	4	9
Dilated medullary tubules	1	0	0	4	3	0	0	0	0	5
Focal loss of tubules	0	1	0	0	2	2	2	1	4	11
Prominent interstitial papillary tissue	0	0	0	0	2	0	0	0	0	8
Papillary mineralization	0	0	0	0	1	0	0	0	0	6
Corticomedullary scarring	0	1	0	0	1	3	0	0	1	10
Cortical cyst(s)[c]	3	3	4	5	2	2	1	1	5	8
Unscheduled										
No. examined	*28*	*29*	*38*	*33*	*42*	*24*	*24*	*27*	*29*	*24*

Table 13 (continued)

	Incidence of finding									
	Males					Females				
	Dietary concentration (ppm)									
	0	30	100	700	4900	0	30	100	700	4900
Dilated/basophilic tubules[a]	2	7	7	10	24	1	1	5	5	5
- grade[b]	[2, 0, 0, 0, 0]	[5, 2, 0, 0 ,0]	[3, 2, 1, 1, 0]	[3, 5, 1, 1, 0]	[2, 4, 8, 6, 4]	[0, 1, 0, 0, 0]	[0, 1, 0, 0, 0]	[3, 1, 0, 1, 0]	[3, 0, 2, 0, 0]	[1, 2, 1, 1, 0]
Dilated/cystic Bowman's capsule	0	2	1	2	12	1	1	1	2	2
Dilated medullary tubules	0	1	2	3	15	1	1	1	1	1
Focal loss of tubules	0	0	2	1	16	0	0	1	0	1
Prominent interstitial papillary tissue	0	0	0	0	9	0	0	1	0	10
Papillary mineralization	0	0	0	0	2	0	0	0	0	1
Corticomedullary scarring	0	0	0	0	0	0	0	0	0	0
Cortical cyst(s)[c]	8	4	5	5	19	2	4	4	7	7

From Green et al. (1986b)

[a] Cortical tubules.

[b] [ ], grade 1, 2, 3, 4 or 5.

[c] Cyst(s) lined by columnar epithelium.

compared with controls. Liver weights, adjusted using body weight as covariate in both sexes in 4900 ppm, were increased at week 26 and at termination (Table 12). The spleen weight was increased in males at 4900 ppm at week 52. Treatment-related non-neoplastic histopathological alterations were confined to the kidneys and the liver in the 4900 ppm group (Table 13). The renal change was basophilic and dilated tubules, sometimes associated with focal tubular loss. The renal lesions occurred in both sexes at 4900 ppm at week 52 and in both sexes at 100, 700 and 4900 ppm at termination and/or in incidental deaths, with increasing incidence with dose levels. In addition, dilated/cystic Bowman's capsules, dilated medullary tubules, focal loss of tubules, prominent interstitial papillary tissue and papillary mineralization were associated with the primary renal change and showed a pattern of increasing incidence with dose levels. Cortical cysts lined by columnar epithelium in the kidney also showed an increased incidence in mice treated at 4900 ppm. There were no other treatment-related non-neoplastic histopathological changes at any dose level.

There were no treatment-related effects in either sex at any dose level on the incidence of any tumour type or on the total number of tumour-bearing animals except for renal tumours in males at 4900 ppm (Table 14). Three male mice bearing renal tumours (one with one adenoma, one with two adenomas and one with carcinoma) were found at 4900 ppm, and another carcinoma was observed in a male of the 700 ppm group. The laboratory historical control incidence of renal cortical tumours is 0.0–2.0%, suggesting that the incidence at 4900 ppm slightly exceeded the historical control incidence. Although the trend test for renal cortical tumours (combined adenoma and carcinoma) was statistically significant ($P = 0.001$) among the groups, the comparison of incidences between the control and 4900 ppm groups was not statistically significant ($P = 0.08$). The trend test between the controls and the 4900 ppm group was marginally significant ($P = 0.046$), and there was no statistical significance between the control and 700 ppm groups ($P = 0.47$). There was a lack of precancerous lesions, including atypical tubules or hyperplastic lesions, in male mice at 4900 ppm. Nevertheless, these tumours are rare in mice and were slightly above the historical control range. Therefore, they were considered to be treatment-related tumours. It is plausible that the continuous stimulation by chronic renal toxicity led to renal tumour development. There were also increases in reticulum cell sarcomas in female mice at 100 ppm and above, but they were not considered treatment related because there was no clear dose–response relationship (2, 2 and 2 at 100, 700 and 4900 ppm, respectively) and they are common tumours in mice. Although reticulum cells were present in many tissues, the increase in reticulum cell sarcomas was not marked.

The NOAEL was 30 ppm (equal to 3.1 mg/kg bw per day), based on an increased incidence of dilated/basophilic renal cortical tubules at 100 ppm (equal to 10.4 mg/kg bw per day). The NOAEL for carcinogenicity in mice was 100 ppm (equal to 10.4 mg/kg bw per day), based on renal cortical tumours at 700 ppm (equal to 75.2 mg/kg bw per day) (Green et al., 1986b).

Rats

Groups of 50 male and 50 female 6- to 7-week-old Sprague-Dawley-derived rats were treated with etofenprox (batch No. ST-103, purity 96.3%) for at least 106 weeks in the diet (in corn oil) at 0, 30, 100, 700 or 4900 ppm (equal to 0, 1.1, 3.7, 25.5 and 186.7 mg/kg bw per day in males and 0, 1.4, 4.8, 34.3 and 249.1 mg/kg bw per day in females, respectively), without correction for purity. Two further subgroups, each with 10 rats of each sex, were killed after 26 or 52 weeks of treatment. The stability and homogeneity of the diet formulations were analysed prior to the start of the study. Concentrations in all diets were analysed during the study. Animals were checked for mortality and clinical signs, including a palpation for tissue masses throughout the study. Rats found dead or killed in extremis were necropsied. Body weights and feed consumption were recorded. Ophthalmic examinations were performed on all animals treated at 0 or 4900 ppm. Blood and urine samples were collected for haematology, clinical chemistry and urinalysis. Clinical chemistry measurements also included T_3 and T_4 activities, but not TSH activities. Subgroups of 10 rats of each sex per dose were sacrificed after 26 and 52 weeks of treatment. All survivors were necropsied after 106 weeks of treatment. At necropsy, blood smears were

Table 14. Summary of renal tumours in a carcinogenicity study in mice with etofenprox

	Males					Females				
	Dietary concentration (ppm)									
	0	30	100	700	4900	0	30	100	700	4900
No. of mice examined[a]	*52*	*52*	*52*	*51*	*52*	*52*	*52*	*52*	*52*	*52*
Not remarkable	17	15	20	12	4	15	25	21	16	9
Autolysis	3	0	0	0	3	1	0	1	1	2
Cortical adenoma	0	0	0	0	1	0	0	0	0	0
Cortical adenoma (two)	0	0	0	0	1	0	0	0	0	0
Cortical carcinoma	0	0	0	1	1	0	0	0	0	0
Reticulum cell sarcoma	0	0	0	0	0	0	0	2	2	2
Lymphosarcoma	1	0	0	1	1	5	0	6	5	4
Lymphoid leukaemia	0	3	0	1	2	3	1	2	1	1
Myeloid leukaemia	1	0	3	0	0	0	0	0	1	0

From Green et al. (1986b)

[a] Number of mice examined includes interim sacrificed and terminal sacrificed.

evaluated. After detailed necropsy, major organs and tissues of animals sacrificed after 26 and 52 weeks and at termination were subjected to histopathological evaluation.

The homogeneity, stability and achieved concentrations of etofenprox in the diets were acceptable. There were no treatment-related clinical findings or increases in palpable tissue masses. The pattern of mortality was comparable among the groups. Survival incidences in the main study groups at termination were 46%, 48%, 46%, 58% and 54% in males and 50%, 34%, 40%, 32% and 52% in females at 0, 100, 300, 700 and 4900 ppm, respectively. The overall feed consumption was reduced in males at 4900 ppm, mainly from week 79 until termination. Females at 4900 ppm showed significantly lower feed consumption at weeks 53–78. The mean body weight gains were decreased in both sexes at 4900 ppm throughout the study. The effect was more severe in the females. The overall weight gain of the males and females was reduced by 24.2% and 34.0%, respectively, compared with controls. There was no effect of treatment on weight gain at lower levels, although males at 700 ppm showed overall weight loss from week 78 to termination (at 78–110 weeks, 88, 38, 15, −45 or −44 g at 0, 30, 100, 700 and 4900 ppm, respectively). There were no treatment-related ophthalmoscopic findings in either sex.

There was a slight treatment-related increase in the clotting times of males at 4900 ppm, relative to the controls, in weeks 16 and 25, which was statistically significant ($P < 0.05$ or 0.01). Significantly higher clotting times were also recorded in males at 700 ppm in week 25 and in all male groups in weeks 51 and 77. However, the individual values at all dose levels in weeks 51 and 77 are considered to be within the normal range of variation. Therefore, an unequivocal treatment-related effect on clotting time is considered to have occurred in males at 4900 ppm in weeks 16 and 25. There was no effect of treatment on clotting times in females at any dose level (Table 15). Reduced haemoglobin concentration and red blood cell count indicating slight anaemia were observed in males at 4900 ppm at week 25. Inconsistent minor inter-group differences in platelet counts and lymphocytes were not considered to be treatment related. There were no treatment-related effects on plasma clinical chemistry, including thyroid hormones, throughout the study. Urinalysis at week 50 revealed increased volumes of more dilute urine compared with controls in males at 700 or 4900 ppm. Some urinary parameters were altered during the study, but not consistently; therefore, these changes were not considered treatment related.

Table 15. Alteration in thrombotest time in the carcinogenicity study in rats with etofenprox

Dietary concentration (ppm)	Sex	Group mean thrombotest time (range) (s)				
		Week				
		16	25	51	77	102
0	Male	33 (28–40)	30 (27–33)	24 (22–26)	26 (20–30)	26 (23–31)
30		31 (29–34)	29 (25–32)	28* (22–32)	28* (24–31)	27 (23–30)
100		33 (30–36)	31 (29–35)	26* (23–29)	30** (26–36)	25 (20–29)
700		35 (26–47)	34** (28–37)	26* (22–28)	30** (29–32)	25 (22–28)
4900		38* (32–47)	38** (33–43)	27** (24–31)	31** (26–36)	25 (21–29)
0	Female	24	22	23	24	23
30		22	22	24	23	23
100		25	22	23	24	22
700		26*	23	23	23	23
4900		25*	22	22	23	22

From Green et al. (1986a)
* $P < 0.05$; ** $P < 0.01$

Liver weights (both absolute and adjusted using body weight) were higher in both sexes at 4900 ppm after 26, 52 and 106 weeks of treatment (Table 16). The thyroid weights of males were increased at 4900 ppm at week 26 and at 700 and 4900 ppm after 106 weeks. The lung weights were increased in males and females at 4900 ppm at week 26 only. Slight increases in kidney weights were evident in females at 4900 ppm at week 26 and in males at 700 and 4900 ppm at week 52. Treatment-related macroscopic lesions were detected in the liver (enlargement or swelling), lungs (pale foci) and thyroid gland of animals at 4900 ppm.

Treatment-related, non-neoplastic histopathological alterations occurred in the liver and thyroid gland (Table 17). Centrilobular hepatocyte enlargement was observed in both sexes at 4900 ppm at termination. Foci or areas of eosinophilic hepatocytes, sometimes associated with vacuolated hepatocytes, occurred in both sexes at 4900 ppm and in males at 700 ppm after 106 weeks. Vacuolated (containing lipid) centrilobular hepatocytes occurred in females at 700 ppm and in both sexes at 4900 ppm after 106 weeks. Males at 4900 ppm also showed a higher incidence of pericholangitis compared with controls. The incidence of cystic follicles in the thyroid gland was increased in females at 4900 ppm. There was also a higher incidence of increased height of the thyroid epithelial cells in females at 4900 ppm at week 26. Other non-neoplastic lesions were considered to be spontaneous in origin.

The incidence of follicular cell adenomas in the thyroid was increased in females at 4900 ppm after 106 weeks of the treatment (Table 18). The incidences of follicular cell carcinoma were not significantly increased in either sex, but there was a statistically significant trend with dose in both sexes for the combined incidences of follicular cell adenoma and carcinoma. The pairwise comparison between the control group and the 4900 ppm group was not statistically significant. The thyroid follicular cell adenoma and combined incidences in males at 4900 ppm showed increased tendency, but were not statistically significant. All other neoplastic alterations in the treated groups were comparable to controls.

The NOAEL was 100 ppm (equal to 3.7 mg/kg bw per day), based on an increased incidence of foci or area of eosinophilic hepatocytes (males only) and vacuolated hepatocytes (females only) and reduced body weight gain (males only) at 700 ppm (equal to 25.5 mg/kg bw per day). The NOAEL for carcinogenic effects was 700 ppm (equal to 34.3 mg/kg bw per day), based on an increased incidence of thyroid follicular cell adenomas and carcinomas combined in females at 4900 ppm (equal to 249.1 mg/kg bw per day) (Green et al., 1986a).

Table 16. Changes in selected organ weights in the carcinogenicity study in rats with etofenprox

Organ	Group mean organ weight (g)									
	Males					Females				
	Dietary concentration (ppm)									
	0	30	100	700	4900	0	30	100	700	4900
Week 26										
Liver	25.1	24.6	23.8	22.2	28.2**	12.6	13.1	11.7	10.8	15.6**
Kidneys	4.77	4.44	4.69	4.68	4.86	2.58	2.77	2.65	2.45	2.91*
Lungs	2.05	2.07	2.28	1.91	2.46**	1.54	1.68	1.59	1.52	1.74*
Thyroid	0.027	0.026	0.027	0.028	0.035*	0.020	0.018	0.020	0.021	0.021
Week 52										
Liver	24.4	25.9	25.9	24.9	31.9**	14.3	14.8	14.6	15.0	16.6*
Kidneys	4.19	4.86	4.60	4.99**	5.05**	3.38	2.94	3.01	3.02	3.14
Lungs	1.99	2.14	2.07	2.03	1.99	1.45	1.52	1.54	1.53	1.60
Thyroid	0.031	0.030	0.035	0.036	0.035	0.025	0.032	0.026	0.025	0.027
Week 106										
Liver	25.5	25.4	25.9	26.2	29.2**	19.2	18.2	19.1	20.0	21.9**
Kidneys	6.52	6.64	6.80	6.70	6.76	4.04	3.99	3.78	3.88	3.80
Lungs	2.51	2.24	2.24	2.27	2.31	1.81	1.67	1.62	1.77	1.75
Thyroid	0.037	0.041	0.040	0.046*	0.050**	0.039	0.032	0.034	0.036	0.041

From Green et al. (1986a)

* $P < 0.05$ (data adjusted using body weight as covariate); ** $P < 0.01$ (data adjusted using body weight as covariate)

2.4 Genotoxicity

Etofenprox has been tested in a battery of genotoxicity studies comprising in vitro gene mutation assays in bacterial and mammalian cells, in vitro and in vivo clastogenicity studies and an in vitro unscheduled deoxyribonucleic acid (DNA) synthesis assay. A summary of the test battery results is shown in Table 19.

Etofenprox was not genotoxic or mutagenic in any of the assays used.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

Two multigeneration studies were performed using rats.

Four groups of 28 male and 28 female 6-week-old Sprague-Dawley-derived rats as the parental F_0 generation were mated twice to provide F_{1a} and F_{1b} generations. Groups of 24 animals of each sex per group, which were selected from the F_{1b} litters, were mated twice to provide F_{2a} and F_{2b} generations. Selected F_{2b} progeny were reared to maturity. The four groups were treated with etofenprox (batch No. ST-103, purity 96.3%) in the diet at nominal concentrations of 0, 100, 700 and 4900 ppm. The diets were fed continuously to the F_0 generation for 25 weeks, to the F_1 generation for 28 weeks and to the F_2 generation for at least 13 weeks from weaning. The F_0 generation animals of both sexes were treated for 10 weeks prior to mating. The etofenprox doses in each generation are shown in Table 20. Reproductive parameters were recorded for each generation and litter. Dietary

Table 17. Selected histopathological changes in carcinogenicity study in rats with etofenprox

Organ and lesion	Males					Females				
	Dietary concentration (ppm)									
	0	30	100	700	4900	0	30	100	700	4900
Week 26										
Number examined	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*
Hepatocyte enlargement	0	0	0	0	9	0	0	0	0	3
Foci or area of eosinophilic hepatocytes	0	0	0	0	0	0	0	0	0	0
Foci or area of basophilic hepatocytes	0	0	0	0	0	0	0	0	0	0
Pericholangitis	0	0	0	1	2	0	0	0	1	0
Thyroid cystic follicles	0	0	0	0	0	0	0	0	0	0
Increased height of thyroid follicular epithelium	0	0	0	0	0	0	0	1	0	5
Week 52										
Number examined	*10*	*10*	*9*	*9*	*10*	*10*	*10*	*10*	*10*	*9*
Hepatocyte enlargement	0	0	0	0	0	0	0	0	0	0
Foci or area of eosinophilic hepatocytes	0	0	0	0	0	0	0	0	0	0
Foci or area of basophilic hepatocytes	0	0	0	0	0	0	0	0	0	0
Pericholangitis	0	0	1	1	2	1	1	2	0	0
Increased height of thyroid follicular epithelium	0	0	1	0	0	0	0	0	0	0
Week 106 + killed/dead										
Number examined	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*
Hepatocyte enlargement	0	0	0	0	5	0	0	0	0	8
Foci or area of eosinophilic hepatocytes	4	11	11	17	22	6	5	6	7	27
Foci or area of basophilic hepatocytes	5	20	15	28	13	24	23	32	26	36
Pericholangitis	14	11	12	14	28	4	4	7	9	8
Vacuolated hepatocytes, centrilobular	6	14	8	10	21	6	7	6	16	24
Thyroid cystic follicles	3	8	8	8	7	4	1	7	6	13
Increased height of thyroid follicular epithelium	0	0	0	0	0	0	0	0	0	0

From Green et al. (1986a)

Table 18. Incidences of follicular cell adenomas/carcinomas in 24-month toxicity and carcinogenicity study in rats of etofenprox

Thyroid tumours	Males					Females				
	Dietary concentration (ppm)									
	0	30	100	700	4900	0	30	100	700	4900
No. of animals examined	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*
Follicular cell carcinoma	0	0	1	3	2	0	0	0	2	1
Follicular cell adenoma	6	6	4	5	11	0	3	2	0	9
Follicular cell adenoma and carcinoma combined	6	6	5	8	13	0	3	2	2	9*

From Green et al. (1986a)
* $P = 0.005$

Table 19. Summary of genotoxicity studies on etofenprox

End-point	Test system; study	Concentration range or dose levels tested	Purity (%)	Batch No.	Result		Reference
					+S9	−S9	
In vitro							
Reverse mutation (Ames test)	*Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, TA1538a)	0, 0 (solvent), 200–3200 µg/plate (±S9)	96.3	ST-103	Negative	Negative	Edwards & Forster (1985)
Assessment of clastogenic activity	Cultured human peripheral lymphocytes; 24 h exposure	24 h: 0 (solvent), 6.25–50 µg/ml (±S9)	96.3	ST-103	Negative	Negative	Bootman, Hodson-Walker & Dance (1985a)
HGPRT gene mutation	Chinese hamster V79 HGPRT$^{+/-}$ cells	0 (solvent), 9.75–156 µg/ml (±S9)	96.3	ST-103	Negative	Negative	Seeburg & Forster (1985a)
Unscheduled DNA synthesis (DNA repair)	HeLa S3 cells	0 (solvent), 9.75–156 µg/ml (−S9)	96.3	ST-103		Negative	Seeburg & Forster (1985b)
		0 (solvent), 2.44–39.0 mg/ml (+S9)			Negative		
In vivo							
Micronucleus test	CD-1 mouse; 24, 48, 72 h sacrifices	24 h: 0, 80, 400, 2000 mg/kg bw	96.3	ST-103	Negative		Bootman, Hodson-Walker & Dance (1985b)
		48 h: 0, 2000 mg/kg bw			Negative		
		72 h: 0, 2000 mg/kg bw			Negative		

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

concentrations, stability and homogeneity were analysed during the study. Major organs and tissues of the F_{1b} parental animals, including male and female sex organs at 0 and 4900 ppm, were examined microscopically. The kidneys of F_0 generation animals treated at 0 or 4900 ppm and F_{1b} generation animals treated at 100 or 700 ppm, the liver and thyroids of F_{1b} generation animals treated at 700 ppm and the thyroids of F_{2b} females treated at all doses were examined microscopically.

Achieved concentrations of etofenprox in formulations were adequate. Red/brown staining of the tray paper under some cages was detected in F_{1a}, F_{1b} and F_{2b} animals at 700 and 4900 ppm. There were six deaths in F_0 and F_{1b} parental rats. A male F_0 rat in the 100 ppm group died at week 10, and two female F_0 generation rats from the same group died or were killed at week 23. These three deaths were not considered treatment related. A male F_{1b} rat in the 700 ppm group died at week 8, a male F_{1b} animal at 4900 ppm was killed in a moribund condition at week 17 and a female F_{1b} rat in the 4900 ppm group died at week 14 due to renal toxicity. The body weight gains were reduced in all generations at 4900 ppm except for F_{2a} males. The water consumption of both sexes at 4900 ppm in the F_{1a}, F_{1b} and F_{2b} generations was increased. In pre-weaning survival, cumulative litter loss in first mating of the F_0 generation was elevated at 4900 ppm (13.9%) compared with the control value (5.4%), but was not statistically significant. The loss was not observed in the second mating of the F_0 generation and first and second generations of the F_{1b} generation.

Table 20. Etofenprox dose in each generation in a multigeneration study in rats

Approximate age (weeks)	Generation	Etofenprox dose (mg/kg bw per day)					
		Males			Females		
		Dietary concentration (ppm)					
		100	700	4900	100	700	4900
5	F_{1a}	14.1	102	744	14.3	104	753
	F_{1b}	13.7	97	702	13.4	95	686
6	F_{2b}	12.9	90	670	13.2	92	670
7	F_{0}	10.6	74	509	10.0	71	481
14	F_{1b}	5.5	37	279	6.5	48	316
16	F_{0}	5.0	37	246	6.6	44	343
16	F_{1a}	5.1	35	267	5.9	44	309
19	F_{2b}	4.3	30	225	5.6	40	302

From Cozens et al. (1985c)

Pup weights in all generations were reduced at 4900 ppm on days 12 and 21 after birth, although they were similar to the control weights at birth. At necropsy, F_{1a}, F_{1b} and F_{2b} generation adults and weanlings showed enlarged, swollen or misshapen kidneys at 4900 ppm, indicating renal toxicity. Ocular defects (small eyes, lenticular opacity, dark eye or intraocular haemorrhage) or subcutaneous haemorrhage was detected in F_{1a} and F_{2a} weanlings (Table 21). They were not considered treatment related because of the low incidence and the lack of a clear dose–response relationship.

Treatment-related changes in organ weights were detected in the kidney, liver and thyroid (Table 22). Kidney weights were increased in all generations at 4900 ppm in both adults and weanlings of both sexes, except for F_{0} females, and in F_{2b} adult females at 700 ppm. The liver weights were increased in all generations at 4900 ppm in adult and weanling animals of both sexes. The increases were also observed in weanlings of the F_{1a} and F_{2b} generations and male weanlings of the F_{1b} generations at 700 ppm. The increases in liver and kidney weights without histopathological changes at 700 ppm were not considered to be treatment related. Thyroid weights were elevated in adult animals of both sexes at 4900 ppm in all generations, except for F_{1b} adult females. Decreased thyroid weights in F_{1b} and F_{2b} weanlings of both sexes in some generations are considered to be a consequence of unusually high control values.

Treatment-related histopathological alterations occurred in the kidneys, liver and thyroid glands of F_{1b} generation adult animals (Table 23). In the kidney, cystic collecting ducts frequently associated with focal medullary fibrosis, mineral deposits, vascular congestion or haemorrhage in the medulla and acute inflammatory cell infiltration in the ducts were increased in both sexes at 4900 ppm. Cortical scarring, basophilic tubules (males) and dilated cortical tubules (females) at 4900 ppm were treatment-related changes. A low incidence of epithelial hyperplasia in the collecting ducts of one male and two females at 4900 ppm was also considered to be treatment related, because spontaneous occurrence of the change is rare for the age of animals examined. Cystic collecting ducts in both the renal medulla and cortex in one female at 700 ppm were not considered to be treatment related. No treatment-related renal alterations occurred in the F_{0} generation adults. In the liver, minimal hepatocyte enlargement in many of the F_{1b} animals was detected. Minimal hepatocyte vacuolation in males at 700 and 4900 ppm was not considered to be treatment related. In the thyroid, slightly increased heights of the columnar epithelium in follicles were observed. There were no treatment-related histopathological alterations in the other tissues examined in F_{1b} animals at 4900 ppm.

Table 21. Incidence of selected clinical or necropsy findings in weanlings

Observation	Incidence of findings							
	First litters (a)				Second litters (b)			
	Dietary concentration (ppm)							
	0	100	700	4900	0	100	700	4900
F_1								
No. examined	*293*	*268*	*271*	*293*	*318*	*230*	*275*	*320*
Kidney lesions[a]	12	0	0	217	0	0	0	196
Ocular lesions	0	0	2	3	0	0	0	0
- small eye	0	0	1	1	0	0	0	0
- lenticular opacity	0	0	1	1	0	0	0	0
- intraocular haemorrhage	0	0	0	1	0	0	0	0
Haemorrhage	0	1[b]	1	1	0	0	0	0
F_2								
No. examined	*250*	*250*	*234*	*253*	*205*	*229*	*240*	*234*
Kidney lesions	0	0	0	157	0	0	0	108
Ocular lesions	0	1	0	0	0	0	0	0
- small eye	0	1	0	0	0	0	0	0
Haemorrhage	0	0	0	3	0	0	0	0

From Cozens et al. (1985c)

[a] Excludes renal pelvic dilatation.

[b] Associated with traumatic injury to cranium.

Table 22. Selected organ weights in adult and weanling rats in a multigeneration study of etofenprox

	Group mean absolute organ weight (g)							
	Males				Females			
	Dietary concentration (ppm)							
	0	100	700	4900	0	100	700	4900
F_0 (adult)								
Kidneys[a]	4.847	4.776	4.790	5.266**	2.865	2.887	2.825	2.987
Thyroid	0.0277	0.0270	0.0306	0.0337**	0.0234	0.0224	0.0245	0.0270*
Liver[a]	26.55	25.97	25.97	30.97**	19.47	19.15	19.32	23.07**
F_{1b} (adult)								
Kidneys[a,b]	4.596	4.808	4.832	5.588**	2.80	2.98	2.95	3.52**
Thyroid[b]	0.0273	0.0314	0.0303	0.0345**	0.0262	0.0257	0.0249	0.0274
Liver[a]	27.93	27.81	27.32	32.40**	18.49	18.88	18.82	22.50**
F_{2b} (adult)								
Kidneys[a]	4.11	3.96	4.15	4.70**	2.187	2.319	2.344*	2.897**
Thyroid	0.0345	0.0342	0.0363	0.0391**	0.0148	0.0180*	0.0180*	0.0188**
Liver[a]	22.36	23.20	23.27	27.93**	11.15	11.26	11.52	13.81**
F_{1a} (weanling)								
Kidneys[a]	0.64	0.64	0.66	1.08**	0.62	0.63	0.66	1.07**

Table 22 (continued)

	Group mean absolute organ weight (g)							
	Males				Females			
	Dietary concentration (ppm)							
	0	100	700	4900	0	100	700	4900
Thyroid	0.0051	0.0050	0.0050	0.0048	0.0052	0.0048	0.0053	0.0050
Liver[a]	2.26	2.37	2.49**	2.82**	2.19	2.20	2.38**	2.75**
F_{1b} (weanling)								
Kidneys	0.659	0.745	0.670	0.969**	0.647	0.748	0.667	0.985**
Thyroid[a]	0.0084	0.0072	0.0078	0.0070*	0.0054	0.0061*	0.0061*	0.0070**
Liver[a]	2.42	2.46	2.56*	2.91**	2.34	2.44	2.44	2.81**
F_{2a} (weanling)								
Kidneys	0.677	0.738	0.746	0.982**	0.662	0.690	0.704	0.957**
Thyroid[a]	0.0053	0.0054	0.0055	0.0052	0.0056	0.0053	0.0055	0.0053
Liver[a]	2.49	2.60	2.59	2.88**	2.32	2.31	2.41	2.75**
F_{2b} (weanling)								
Kidneys	0.734	0.712	0.738	0.955**	0.697	0.684	0.743	0.944**
Thyroid	0.0095	0.0067**	0.0066**	0.0059**	0.0080	0.0067**	0.0067**	0.0065**
Liver[a]	2.55	2.64	2.74**	3.06**	2.44	2.51	2.63**	2.82**

From Cozens et al. (1985c)
* $P < 0.05$; ** $P < 0.01$
[a] Covariate adjusted to body weight.
[b] Log-transformed data.

Table 23. Selected histopathological alterations in F_{1b} adult rats in a multigeneration study of etofenprox

Organ and findings	Group mean value							
	Males				Females			
	Dietary concentration (ppm)							
	0	100	700	4900	0	100	700	4900
Kidneys								
No. examined	*24*	*24*	*23*	*23*	*24*	*24*	*24*	*23*
Cystic collecting ducts								
- medulla	0	0	0	17	0	0	1	19
- cortex/medulla	0	0	0	5	0	0	0	4
Focal fibrosis[a]	0	0	0	9	0	0	0	17
Vascular congestion/haemorrhage[a]	0	0	0	4	0	0	0	5
Mineral deposits[a]	0	0	0	3	0	0	0	17
Acute inflammatory cells in ducts	0	0	0	7	0	0	0	4
Cortical scarring	0	0	0	14	0	0	0	11
Epithelial hyperplasia	0	0	0	1	0	0	0	2
Dilated tubules[b]	0	0	0	3	2	2	1	7
Glomerulonephritis[c]	2	1	3	5	1	0	0	3

Table 23 (continued)

Organ and findings	Group mean value							
	Males				Females			
	Dietary concentration (ppm)							
	0	100	700	4900	0	100	700	4900
Liver								
No. examined	*24*	*0*	*23*	*23*	*24*	*0*	*24*	*23*
Hepatocyte enlargement[d]	0	—	0	18	0	—	0	9
Hepatocyte vacuolation								
- moderate	1	—	1	1	0	—	0	0
- minimal	2	—	5	4	0	—	1	0
Thyroid								
No. examined	*24*	*0*	*23*	*23*	*24*	*0*	*24*	*23*
Increased height of columnar epithelium	0	—	0	6	0	—	0	0

From Cozens et al. (1985c)
[a] Medullary.
[b] Cortical.
[c] Early progressive.
[d] Minimal.

The NOAEL for parental toxicity was 700 ppm (equal to 37 mg/kg bw per day), based on the occurrence of reduced weight gain, increased kidney, liver and thyroid weights and histopathological findings in the liver, kidneys and thyroid at 4900 ppm (equal to 246 mg/kg bw per day). The NOAEL for reproductive effects was 4900 ppm (equal to 246 mg/kg bw per day), the highest dose tested. The NOAEL for offspring toxicity was 700 ppm (equal to 37 mg/kg bw per day), based on the occurrence of increased kidney weights of females in the F_{2b} generation at 4900 ppm (equal to 246 mg/kg bw per day) (Cozens et al., 1985c).

A study of the perinatal and postnatal periods of the rat with rearing to maturation of the F_1 offspring was conducted as a modified reproductive study. Groups of 25 female 8- to 9-week-old Sprague-Dawley rats were treated by gavage with etofenprox (batch No. ST-103, purity 96.3%) suspended in aqueous methylcellulose at nominal dose levels of 0 (vehicle only), 12.5, 250 and 5000 mg/kg bw per day from day 17 of gestation until day 21 postpartum, inclusive. The dose volume was 20 ml/kg bw. The concentrations, stability and homogeneity of the dosing formulations were analysed during the study. Clinical signs, body weights and feed consumption were measured throughout the study. The F_1 progeny of F_0 females were examined externally and internally for abnormalities. During lactation, all litters were checked for their development. Blood samples were withdrawn from three F_1 pups of each sex per litter on day 22, and activated partial thromboplastin time, prothrombin time, packed cell volume, haemoglobin, red blood cells, mean corpuscular haemoglobin, mean corpuscular volume and platelets were each determined in one pup of each sex per litter. These pups and F_0 parental females were then sacrificed. The sex of the pups was confirmed by gonadal inspection. The kidneys of pups treated at 5000 mg/kg bw per day and representative control pups were weighed and examined microscopically. One pup of each sex was selected for further study on day 21 postpartum. Selected F_1 progeny (25 of each sex per group) were maintained untreated and weighed weekly until termination. The feed consumption was measured throughout, with the exception of the mating period and during gestation in females. Water consumption was measured for 1 week on three occasions. The F_1 animals were regularly observed for signs of abnormal behaviour. Reproductive

capability was assessed from approximately 84 days of age by 1:1 matings of F_1 animals for 14 days. During the mating period, females were weighed every 2 days until sperm were observed in a vaginal smear or a plug (= day 0 of gestation). Daily vaginal smears were also evaluated to determine marked anomalies of the estrous cycle. The F_2 progeny of F_1 females were examined as soon as possible after the completion of parturition, counted, sexed, weighed and examined for external abnormalities. All F_2 progeny and F_1 parental animals were killed on, or shortly after, day 21 postpartum and examined for internal and external abnormalities. The kidneys of parental animals in the F_1 generation were weighed and examined microscopically.

Concentrations and homogeneity in the formulations were acceptable. Salivation and occasional red-brown staining around the mouth were observed in F_0 females treated at 250 or 5000 mg/kg bw per day (Table 24). In addition, most rats of the 5000 mg/kg bw per day group showed yellow staining of the fur in the anogenital region towards the end of the treatment period. There were no treatment-related clinical signs in rats of the 12.5 mg/kg bw per day group.

A slight decrease in body weight gain (13.4%) was observed at 5000 mg/kg bw per day from day 17 to day 20 of gestation. No treatment-related effects on feed consumption were observed at any dose level other than 5000 mg/kg bw per day, at which dose feed consumption was 7.1% lower during the final week of lactation. F_1 pup mortality at 5000 mg/kg bw per day increased markedly. On day 21, the cumulative high-dose F_1 pup mortality from day 4 to day 21 was higher than that of the controls (26.1% versus 2.7% in controls). Increased mortality was accompanied by clinical signs of skin darkening around the nose (confirmed at necropsy as subcutaneous haemorrhage), tremors and general motor incoordination at 5000 mg/kg bw per day. F_2 pup body weight at 5000 mg/kg bw per day was reduced from day 8, and the effect persisted until day 21. Fetal body weights (combined males and females) on days 12 and 21 in the F_0 generation and day 4 in the F_1 generation were reduced.

There was no effect of treatment on development in the F_1 generation at any dose level. In haematological examination of 22-day-old weanlings of the 5000 mg/kg bw per day group, prothrombin time and activated thromboplastin time were lower than the control values in males, and platelet count was increased in females. Necropsy of the F_1 generation pups dying prior to weaning and surplus pups killed at weaning revealed a high proportion in the 5000 mg/kg bw per day group with enlarged and often pale and scarred kidneys. One pup from each of the lower dose levels also showed enlarged kidneys. Kidney weights at 5000 mg/kg bw per day were high in both sexes. Treatment-related histopathological renal alterations at 5000 mg/kg bw per day were cystic collecting ducts, occasionally restricted to the medulla, but more frequently extending into the cortex, acute inflammatory cells in the collecting ducts, early cortical scarring and mineral casts in the medulla. At lower dose levels with gross renal lesions, pelvic dilatation and focus of lymphocytic infiltration in the cortex were observed at 12.5 and 250 mg/kg bw per day, respectively. These changes were not treatment related.

During the F_1 pre-mating development phase, blood-stained urine occurred at 5000 mg/kg bw per day. A slightly reduced body weight was noted in both sexes at 5000 mg/kg bw per day during lactation. Water consumption in both sexes at 5000 mg/kg bw per day was increased. The time of vaginal opening and the behavioural development of F_1 progeny were unaffected by treatment at all dose levels. The gestation and litter parameters were not affected by treatment. Although mean pup body weight at 5000 mg/kg bw per day was slightly depressed throughout lactation, the depressions were considered to be related to the increased litter size.

Necropsy of F_1 adults at 5000 mg/kg bw per day revealed marked renal effects of pale, scarred and misshapen kidney cortices, which correlated histologically with cystic collecting ducts, focal medullary fibrosis, cortical scarring and foreshortened papilla at 5000 mg/kg bw per day (Table 25). These renal lesions were not evident at lower dose levels. Relative kidney weights were also increased at 5000 mg/kg bw per day. Macroscopic changes in F_2 weanlings indicate no effect of treatment.

Table 24. Clinical signs of F_0 generation in perinatal and postnatal periods of maturation study in rats

Clinical signs	Dose (mg/kg bw per day)			
	0	12.5	250	5000
No. of rats examined	*25*	*25*	*25*	*25*
Increased salivation	0	0	15	25
- sign occurring on ≥ 5 days	—	—	1	22
Red staining around the mouth	0	0	8	17
- sign occurring on ≥ 5 days	—	—	0	2
Wet/yellow-stained fur around the anogenital region	0	0	0	15
- sign occurring on ≥ 5 days	—	—	—	0

From Cozens et al. (1985b)

Table 25. Relative kidney weights and selected histopathological renal alterations in F_1 adult rats treated with etofenprox

Parameter	Group mean value							
	Males				Females			
	Dose (mg/kg bw per day)							
	0	12.5	250	5000	0	12.5	250	5000
Relative kidney weight (%)	4.027	4.167	4.024	4.569**	2.355	2.365	2.452	2.742**
No. examined (kidneys)	23	19	23	21	23	19	23	21
Cystic collecting ducts								
- medulla	0	0	0	17	0	0	0	17
- cortex/medulla	0	0	0	4	0	0	0	4
Focal fibrosis in medulla	0	0	0	13	0	0	0	8
Vascular congestion/ haemorrhage in medulla	0	0	0	5	0	0	0	5
Mineral casts in medulla	0	0	0	2	0	0	0	8
Acute inflammatory cells in ducts	0	0	0	6	0	0	0	7
Cortical scarring	0	0	0	18	0	0	0	13
Papilla foreshortened	0	0	0	10	0	0	0	12
Papillary necrosis	0	0	0	3	0	0	0	0
Pyelitis	0	0	0	1	0	0	0	5

From Cozens et al. (1985b)
** $P < 0.01$

The NOAEL for parental toxicity was 250 mg/kg bw per day, based on the clinical changes suggesting renal toxicity in F_0 females at 5000 mg/kg bw per day. The NOAEL for offspring toxicity was 250 mg/kg bw per day, based on the occurrence of tremor, subcutaneous haemorrhage, reduced weight gain, increased water consumption, blood-stained urine, increased pup mortality and histopathological alterations in the kidneys of F_1 generation progeny at 5000 mg/kg bw per day (Cozens et al., 1985b).

(b) Developmental toxicity

Rats

In an oral developmental/fertility study, groups of 24 male and 24 female 7-week-old CD rats were treated with etofenprox (batch No. ST-103, purity 96.3%) suspended in aqueous methylcellulose at a nominal dose level of 0 (vehicle only), 12.5, 250 or 5000 mg/kg bw per day by gavage (dose volume 20 ml/kg bw). Males were treated daily for 9 weeks prior to mating, throughout the cohabitation period and during the gestation period of the females. Females were treated daily for 2 weeks prior to mating, throughout cohabitation and up to day 7 of gestation. The concentration, stability and homogeneity of the formulations of test compound were analysed. Animals were paired within each group for the 14-day mating period. Daily vaginal smears were examined for 3 days before the start of cohabitation and throughout the mating period until sperm were observed. Clinical signs, body weights and feed consumption were measured during the study. Males were sacrificed at the end of the gestation period of the mated females and subjected to postmortem examination of major organs and tissues. The testes of males that failed to inseminate a female were preserved for subsequent histological evaluation. Maternal females were necropsied on day 20 of gestation and subjected to postmortem examination of major organs and tissues. The uterus was examined for the number and position of live young, the number and distribution of embryonic/fetal deaths, individual fetal weights and gross fetal abnormalities. The ovaries were examined for the number of corpora lutea. Embryonic/fetal deaths were classified as early (placental remnants visible only) or late (both placental and embryonic remnants visible). Live fetuses were weighed and examined for external malformations. Approximately 50% of the fetuses from each litter were fixed in Bouin's fluid for examination of visceral abnormalities. The remaining fetuses were retained for skeletal examination.

The concentrations and homogeneity of the etofenprox formulations were acceptable. There were no deaths at any dose level during the study. Salivation and brown staining around the mouth post-dosing were observed as minor treatment-related clinical signs at all dose levels. Salivation and red staining around the mouth without consistency at 12.5 and 250 mg/kg bw per day in the F_0 generation were considered to be a reaction to stimulation at gavage, but not adverse effects of etofenprox. Staining/wetness in the anogenital region, white crystalline appearance to faeces and matted/damp fur were evident in males only at 5000 mg/kg bw per day. The feed consumption and body weight gain of both sexes were not adversely affected by the treatment. No treatment-related changes were observed in the pre-mating, cohabitation or gestation indices. There were no treatment-related effects at any dose level on median pre-coital time, pregnancy incidence or implantation number. A slight but significant increase in pre-implantation loss was observed at 5000 mg/kg bw per day. The number of early resorptions per litter at 5000 mg/kg bw per day was slightly higher, but the overall post-implantation loss was similar to that in the controls. The number of live fetuses per litter and the litter weight at 5000 mg/kg bw per day were marginally low, but not significantly. Fetal weight and sex ratio were unaffected by treatment. No abnormalities were detected in histology of the testes.

No treatment-related malformations were observed in fetuses of any treatment group. The incidences of visceral and skeletal anomalies and skeletal variants did not indicate an effect of treatment at any dose level. Although the incidence of skeletal anomalies was higher than the control incidence in all treated groups, the nature of the anomalies was similar to those in the controls, the higher incidences were not dose related and they were not statistically significantly different from the controls. Therefore, the differences are considered to be spurious.

The NOAEL for reproductive and fertility effects was 5000 mg/kg bw per day, based on the absence of reproductive and fertility effects at the highest dose level tested. The NOAEL for parental systemic toxicity was 250 mg/kg bw per day, based on the occurrence of clinical signs (staining/wetness in the anogenital region, white crystalline appearance to faeces and matted/damp fur) in males at 5000 mg/kg bw per day. The NOAEL for developmental effects was 5000 mg/kg bw per day, the highest dose tested (Cozens et al., 1985a).

Table 26. Clinical signs of F_0 generation in a developmental study in rats

Clinical signs	Dose (mg/kg bw per day)			
	0	12.5	250	5000
No. of rats examined	*35*	*35*	*35*	*35*
Increased salivation	0	7	15	32
- sign occurring on ≥ 5 days	—	0	0	2
Red staining around the mouth	0	2	2	14
- sign occurring on ≥ 5 days	—	0	1	0
Wet/yellow-stained fur around the anogenital region	0	0	0	30
- sign occurring on ≥ 5 days	—	—	—	20

From Cozens, Hughes & Anderson (1985)

A modified developmental/reproductive toxicity study in rats was also conducted. To evaluate the behavioural development and reproductive capabilities following in utero exposure, groups of 35 female 8- to 9-week-old Sprague-Dawley rats were treated with etofenprox (batch No. ST-103, purity 96.3%) suspended in aqueous methylcellulose at 0 (vehicle only), 12.5, 250 or 5000 mg/kg bw per day by gavage from day 6 to day 17 of gestation. The gavage dose volume was 20 ml/kg bw. On day 20 of gestation, 21–24 females per group were killed to examine effects on embryo and fetal development. Approximately 50% of the fetuses from each litter were processed for skeletal examination. Abnormalities in fetuses were classified as malformations, anomalies or variations. The remaining 11–14 females per group were allowed to litter normally and rear their young. One pup of each sex of F_0 progeny was selected for further study on day 21 postpartum, at which time excess pups and F_0 parental females were sacrificed and examined externally and internally for abnormalities. Selected F_1 progeny were maintained untreated, and signs of abnormal behaviour, body weights and feed consumption were recorded. The onset of vaginal opening was monitored, and the developmental/behavioural parameters were examined from 6 weeks of age. F_1 rats approximately 84 days old were mated (1:1) to evaluate reproductive capabilities. All F_2 progeny and F_1 parental rats were killed shortly after day 21 postpartum and examined for internal and external abnormalities.

The concentrations and homogeneity of the formulations were acceptable. In the treated F_0 female parental animals, a dose-related increase in incidence of salivation and red-brown staining around the mouth following dosing was observed at all dose levels (Table 26). In addition, wet, yellow staining of the fur in the anogenital region was observed in most animals treated at 5000 mg/kg bw per day towards the end of the treatment period. Body weight gain was marginally reduced at 5000 mg/kg bw per day during gestation. Feed consumption was unaffected by treatment at all dose levels. There were no treatment-related macroscopic findings in F_0 females except for a low incidence of minor skin lesions at 5000 mg/kg bw per day.

There were no treatment-related effects on the F_1 generation during pre-mating at all dose levels. The F_1 maternal body weight gain at 5000 mg/kg bw per day was 7% lower during gestation, but the decrease was not statistically significant. The effect did not persist during lactation. The F_1 adult progeny and the F_2 weanlings did not reveal any treatment-related gross lesions at any dose level.

The NOAEL for parental toxicity was 250 mg/kg bw per day, based on the occurrence of minor clinical signs in the F_0 generation and slightly reduced weight gain during gestation in the F_1 generation at 5000 mg/kg bw per day. The NOAEL for reproductive toxicity was 5000 mg/kg bw per day, based on the absence of effects at 5000 mg/kg bw per day. The NOAEL for developmental toxicity was 5000 mg/kg bw per day, the highest dose tested. The NOAEL for offspring toxicity was 250 mg/kg bw per day, based on the occurrence of minimally reduced weight gain during gestation in the F_1 generation at 5000 mg/kg bw per day. Etofenprox was not teratogenic (Cozens, Hughes & Anderson, 1985).

Rabbits

Two developmental toxicity studies in rabbits were performed.

Groups of 16 or 17 mated 13- to 16-week-old female New Zealand White rabbits were treated by gavage with etofenprox (batch No. ST-103, purity 96.3%) suspended in aqueous methylcellulose at a nominal dose level of 0 (vehicle only), 10, 50 or 250 mg/kg bw per day from day 6 through 18 of gestation inclusive. The dose volume was 1 ml/kg bw. The New Zealand White rabbits were purchased from three different suppliers. The achieved concentration and homogeneity of the dosing formulations were analysed. Clinical signs, body weights and feed consumption were recorded. The animals were sacrificed on day 29 of gestation to determine parental, embryonic and fetal abnormalities. The ovaries were examined for the number of corpora lutea. Live fetuses were examined for visceral and skeletal abnormalities.

The concentrations and homogeneity of the dosing formulations were acceptable. There were no deaths during the study at any dose level. Treatment-related maternal clinical signs were reduced faecal output and reduced water consumption at 250 mg/kg bw per day. There were no treatment-related clinical signs at lower dose levels. Body weight depression accompanied by decreased feed consumption was observed from day 6 to day 10 of gestation at 250 mg/kg bw per day (Table 27). Thereafter, the animals gained weight, but at termination on day 29, the overall weight gain from day 6 to day 29 was 29.5% lower than the control gain. Two rabbits at this dose level showed particularly marked weight loss (> 500 g) throughout the treatment period. Reduced body weight gain was observed during days 6–8 of the treatment at 50 mg/kg bw per day, and overall weight gain from day 6 to day 29 was reduced by 11.3%. The changes in body weights at 50 mg/kg bw per day on days 6–8 were spurious findings, as body weight gains were recovered in 2 days or so. Feed consumption at 50 mg/kg bw per day was also slightly reduced from day 6 to day 9, but the effect was not statistically significant. The weight gain and feed consumption of the group treated at 10 mg/kg bw per day were not affected by treatment.

There were no treatment-related gross findings at necropsy. One animal at 10 mg/kg bw per day and two animals at 250 mg/kg bw per day showed evidence of abortion, and a further control animal and one at 10 mg/kg bw per day showed total resorption. The incidence of early resorptions was increased at 250 mg/kg bw per day and, in conjunction with aborted fetuses, led to increased post-implantation loss and reduced litter size and weight at 250 mg/kg bw per day, but all of these changes were not statistically significant (Table 28). There were no treatment-related effects on the nature or incidence of fetal malformations or anomalies at any dose level.

The NOAEL for maternal toxicity was 50 mg/kg bw per day, based on the overall reduced body weight gain and feed consumption at 250 mg/kg bw per day. The NOAEL for developmental toxicity was 50 mg/kg bw per day, based on the occurrence of slightly increased post-implantation loss at 250 mg/kg bw per day. Etofenprox was not teratogenic in rabbits (Bottomley, 1985).

A second developmental toxicity study in rabbits was performed in which groups of 22 mated 5-month-old female New Zealand White rabbits were treated by gavage with etofenprox (batch No. 21088, purity 96.68%) suspended in aqueous methylcellulose at a nominal dose level of 0, 30, 100 or 300 mg/kg bw per day from day 6 through day 28 of gestation inclusive. The dose volume was 1 ml/kg bw. The concentration, stability and homogeneity of the dosing formulations were analysed. Mortality, clinical signs, body weights and feed consumption were recorded. The animals were sacrificed on day 29 of gestation to determine parental, embryonic and fetal abnormalities. The ovaries were examined for the number of corpora lutea. Live fetuses were examined for visceral and skeletal abnormalities.

The concentrations and homogeneity of the dosing formulations were acceptable. Six animals (one, one and four does treated at 30, 100 and 300 mg/kg bw per day, respectively) died or were euthanized

Table 27. Summary of group mean feed consumption and body weight gain for all animals in the rabbit developmental study of etofenprox

	Dose (mg/kg bw per day)			
	0	10	50	250
Mean feed consumption (g/day)				
Days 1–5	194	184	181	171
Days 6–7	212	193	184	125***
Days 8–9	214	192	194	133***
Days 10–13	194	178	196	150*
Days 14–18	186	191	194	146
Days 19–22	182	193	180	164
Days 23–28	135	142	133	123
Mean body weight change (g)				
Days 1–6	129	134	96	86
Days 6–8	70	66	23*	−41**
Days 6–10	124	105	107	−5**
Days 6–14	249	223	252	113
Days 6–19	361	382	347	173
Days 6–23	442	438	430	269
Days 6–29	533	565	473	376

From Bottomley (1985)
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 28. Summary of litter parameters, including abortions and total resorptions, for all pregnant animals in the rabbit developmental toxicity study with etofenprox

Parameter	Group mean value			
	Dose (mg/kg bw per day)			
	0	10	50	250
Number mated	17	17	16	17
Number killed prior to treatment	1	1	0	1
Number with total resorption	1	1	0	0
Number aborted	0	1	0	2
Number not pregnant	1	0	0	1
Number with live litter on day 29	14	14	16	13
Number of corpora lutea	12.2	11.5	11.4	11.4
Number of implantation sites	10.3	9.1	10.0	9.7
Pre-implantation loss (%)	16.1	19.9	11.1	12.8
Number of resorptions (mean/litter)				
- early	0.5	1.0	0.5	1.3
- late	1.3	0.4	0.6	0.5
- aborted	0.0	0.3	0.0	1.3
- total	1.8	1.7	1.1	3.2
Post-implantation loss (%)[a]	17.6 (11.7)	20.7 (9.3)	11.8	33.6 (23.3)
Number of live fetuses (mean/litter)[a]	8.5 (9.1)	7.4 (8.4)	8.9	6.5 (7.5)
Sex ratio (% males)	52.4	46.8	50.1	50.7
Litter weight (g)	368.8	366.9	371.4	308.2
Male + female fetal weight (g)	41.5	43.9	43.1	42.3

From Bottomley (1985)
[a] ( ) includes data from animals with viable young on day 29.

Table 29. Summary of maternal body weight, body weight gain and feed intake in developmental study of etofenprox in rabbits

	Dose (mg/kg bw per day)			
	0	30	100	300
Body weight gain (g)				
Days 0–4	148.1	106.5	156.38	78.05
Days 4–6	17.57	35.95	12.19	48.71
Days 6–9	25.52	12.25	5.43	−56.33
Days 9–12	−11.76	50.65*	8.62	1.33
Days 12–15	76.52	46.20	50.14	6.57**
Days 15–18	70.71	72.50	55.29	−13.55**
Days 18–21	48.81	61.10	41.48	31.95
Days 21–24	32.33	46.85	42.48	−19.75
Days 24–27	−5.29	−14.10	−5.45	−75.39
Days 27–29	11.48	17.16	12.05	−37.06
Body weight (kg)				
Day 0	3.68	3.71	3.65	3.68
Day 6	3.85	3.85	3.82	3.81
Day 29	4.10	4.11	4.04	3.70**
Food intake (g/kg bw)				
Days 4–6	165.7	171.2	172.4	170.8
Days 6–29	152.3	155.7	146.5	123.5*

From Fisher (2000)
* $P < 0.05$; ** $P < 0.01$

due to moribund condition. One doe that died at 100 mg/kg bw per day had no remarkable clinical observations. One doe in the 30 mg/kg bw per day group aborted, and two does in the 300 mg/kg bw per day group aborted. These abortions were observed at a late stage of the pregnancies. No remarkable clinical observations were reported in one doe that aborted at 30 mg/kg bw per day. Treatment-related clinical signs were thinness and few or no faeces, confined to two does that aborted in the 300 mg/kg bw per day group. Treatment-related gross findings at necropsy in maternal animals (which died or were euthanized) were gastrointestinal distension and mucosal irritation in animals at 300 mg/kg bw per day. Females at 300 mg/kg bw per day showed body weight decreases accompanied by reduced feed consumption from day 6 to day 29 (Table 29). There were no treatment-related gross lesions at necropsy in the animals that survived to day 29. All animals that died or were killed prematurely were pregnant.

There were no effects of treatment on pregnancy incidence, gravid uterus weight, number of corpora lutea, pre-implantation loss, number of live fetuses or sex ratio at any dose level (Table 30). An increase in post-implantation loss due to an increased incidence of litters with resorption was observed at 300 mg/kg bw per day. The number of live fetuses per litter at 300 mg/kg bw per day was comparable to that of the control group. The fetal body weights in both sexes at 300 mg/kg bw per day were significantly reduced.

There were no increases in external, visceral or skeletal malformations in any treated group compared with controls. An increased incidence of fetal unossified talus at 300 mg/kg bw per day was considered to be intrauterine growth retardation (Table 31). The incidence of 13th full rib was slightly higher than the maximum historical control incidence of 42%, but as the difference was numerically small and the litter incidence at 300 mg/kg bw per day (88%) was comparable to the

Table 30. Summary of litter parameters in developmental toxicity study with etofenprox in rabbits

Parameter	Group mean value			
	Dose (mg/kg bw per day)			
	0	30	100	300
Number mated	22	22	22	22
Number pregnant	21	20	21	21
Number aborted	0	1	0	3
Number dying	0	0	1	2
Number with live litter on day 29	21	19	20	17
Gravid uterus weight (g)	472.6	522.4	503.2	436.7
Number of corpora lutea	9.7	10.1	10.3	10.4
Number of implantation sites	8.1	8.7	9.0	9.3
Pre-implantation loss (%)	16.1	12.9	14.5	10.9
Resorptions (% mean/litter)				
- early	0.2	0.1	0.3	0.6
- late	0.2	0.1	0.1	0.4
- total	0.4	0.2	0.4	1.0
Total number of dead fetuses	0	0	0	0
Post-implantation loss (% mean/litter)	4.3	1.5	3.8	10.1
Total number of live fetuses	162	163	172	141
Number of live fetuses (mean/litter)	7.7	8.6	8.6	8.3
Sex ratio (% males)	58	52	46	45
Fetal weight[a] (g)				
- male	41.32	43.37	42.47	35.92**
- female	41.62	43.13	41.16	35.11**
- mean (male + female)	41.70	43.45	41.94	35.66**

From Fisher (2000)
** $P < 0.01$
[a] Covariate adjusted.

control group litter incidence (86%), it is considered not to be treatment related. Unossified 5th sternebra was significantly higher ($P < 0.05$) at 30 and 100 mg/kg bw per day, but not at 300 mg/kg bw per day. As there was no dose–response relationship and as all incidences for unossified 5th sternebra were within the historical control range of 4.5–17%, the differences are considered not to be related to treatment with etofenprox.

The NOAEL for maternal toxicity was 100 mg/kg bw per day, based on the occurrence of reduced weight gain and feed consumption, mortality and abortions at 300 mg/kg bw day. The NOAEL for developmental toxicity was 100 mg/kg bw per day, based on the occurrence of slightly increased post-implantation loss and intrauterine growth retardation (increased incidence of 13th full rib) at 300 mg/kg bw per day. Etofenprox was not teratogenic in rabbits (Fisher, 2000).

When the results of these two developmental toxicity studies in rabbits are combined, the overall NOAEL for developmental toxicity and maternal toxicity was 100 mg/kg bw per day, based on the occurrence of reduced maternal body weight gain and feed consumption (during the early dosing period, gestation day 6), abortions, mortality and slightly increased post-implantation loss and intrauterine growth retardation at the high dose of 250 mg/kg bw per day.

Table 31. Incidence of external, visceral and skeletal variations in a developmental toxicity study of etofenprox in rabbits

Parameter	Incidence (%)			
	Dose (mg/kg bw per day)			
	0	30	100	300
Number of litters evaluated	*21*	*19*	*20*	*17*
Number of fetuses evaluated	*162*	*163*	*172*	*141*
Incidence of external variations				
- litter	0	0	0	0
- fetal	0	0	0	0
Incidence of visceral variations				
- litter	76	63	85	59
- fetal	23	18	20	16
Incidence of skeletal malformations				
- litter	100	100	100	100
- fetal	80	79	76	88
Fetal incidence of unossified talus	0	0	2.3	4.3*
Fetal incidence of unossified 5th sternebra	4.3	12*	16*	9.2
Fetal incidence of 13th full rib	40	42	33	56*

From Fisher (2000)
* $P < 0.05$

2.6 Special studies

(a) Acute oral neurotoxicity

In an acute neurotoxicity study, groups of fasted 7- to 8-week-old Sprague-Dawley rats (Crl:CD(SD)IGS BR) (10 of each sex per dose) were given a single oral gavage dose of etofenprox (batch No. 87031, purity 99%) in 1.0% methylcellulose in reverse osmosis water at a dose of 0, 25, 125, 500 or 2000 mg/kg bw and observed for 15 days. The dose volume was 10 ml/kg bw. The concentrations, stability and homogeneity of the dosing formulations were analysed. Morbidity/mortality, clinical signs, body weights and feed consumption were measured. Neurobehavioural assessment (functional observational battery [FOB] and motor activity testing) was performed on 10 rats of each sex per group prior to dosing and at days 1 (approximately 5 hours after treatment on day 1; the estimated time of peak effect), 8 and 15 following etofenprox exposure. At study termination, all rats were killed and perfused in situ for neuropathological examination. Of the perfused animals, six animals of each sex per group from the control and highest-dose groups were subjected to histopathological evaluation of brain and peripheral nervous system tissues.

The concentrations, stability and homogeneity of the dosing formulations were acceptable. There were no treatment-related effects on mortality, clinical signs, body weight, brain weight or gross and histological pathology or neuropathology. FOB and motor activity testing revealed no treatment-related effects.

The NOAEL for systemic and acute neurotoxicity was 2000 mg/kg bw per day, the highest dose tested, based on the absence of adverse effects, including functional observations and histopathology, at this dose level (Smith, 2002).

(b) Subchronic neurotoxicity

In a 13-week neurotoxicity study, etofenprox (batch No. 87137, purity 99%) was administered to 10 Crl:CD(SD)IGS BR Sprague-Dawley rats of each sex per group at a dose level of 0, 2500, 5000 or 10 000 ppm in the diet (equal to 0, 149, 299 and 604 mg/kg bw per day for males and 0, 174, 350 and 690 mg/kg bw per day for females, respectively) for 13 weeks. Corn oil was used as the vehicle to mix the etofenprox with diet. The concentrations, stability and homogeneity of etofenprox in the diets were analysed. Morbidity/mortality, detailed and daily clinical examinations, body weights and feed consumption were recorded. Neurobehavioural assessment (FOB and motor activity testing) was performed on 10 rats of each sex per group at pretreatment and at 1, 5, 9 and 13 weeks. After 13 weeks of treatment, blood samples were taken for haematological evaluation. At study termination, 10 rats of each sex per group were killed and perfused in situ for neuropathological examination. Of the perfused animals, six rats of each sex from the control and 10 000 ppm groups were subjected to microscopic evaluation of brain and peripheral nervous system tissues. Brains were not weighed.

The concentrations, homogeneity and stability of the treated diets were acceptable. There were no deaths during the study. There were no treatment-related clinical signs of toxicity. Body weight and body weight gain of males were not affected by the treatment. The terminal mean body weight of females that received 10 000 ppm in the diet was 89% of the control value (non-significant). The decreased body weights of high-dose females were not considered an adverse effect, because the decrease was small and there was a lack of statistical significance. The overall body weight gain was statistically significantly lower in females at 10 000 ppm compared with controls. There were no treatment-related ocular lesions. In the 10 000 ppm group, females showed slightly lower red blood cell count, haemoglobin concentration and haematocrit, and both sexes showed slightly shorter prothrombin times after 13 weeks of treatment. These effects were not considered as adverse, because the changes were minor. Treatment-related effects observed in the qualitative observations of the FOB were confined to an increased incidence of stained fur in females at 10 000 ppm at weeks 9 and 13. As it was an inconsistent and isolated observation during the weekly detailed examinations, stained fur is considered not to be an adverse effect of treatment. Abnormal gait, described as tiptoe in females at weeks 5, 9 and 13, showed no clear dose–response relationship. There were no treatment-related effects on other qualitative FOB parameters.

There were no treatment-related findings at necropsy at any dose level. The absolute and relative liver weights of males at all dose levels and females at 10 000 ppm showed dose-related increases. The increases in liver weights were not considered to be an adverse effect, as there was no correlating liver pathology. The absolute and relative thyroid weights were increased in both sexes at 10 000 ppm, but not statistically significantly. Macroscopic examination of the treated animals was unremarkable. There were no treatment-related histopathological alterations in the central or peripheral nerve system tissues in animals at 10 000 ppm.

The NOAEL for systemic toxicity was 10 000 ppm (equal to 604 mg/kg bw per day), the highest dose tested. The NOAEL for neurotoxicity was 10 000 ppm (equal to 604 mg/kg bw per day), based on the absence of adverse effects on FOB and motor activity and histopathology at the highest dose level. Etofenprox is not considered neurotoxic in adult rats (Smith, 2003b).

(c) Developmental neurotoxicity

The dose levels for the developmental neurotoxicity study were based on the results of an oral dose range–finding study in rats in which five groups of eight animals each were treated with etofenprox in the diet at a nominal concentration of 0, 700, 1400, 2800 or 4900 ppm from day 6 of gestation to day 21 of lactation. Mild maternal toxicity, increased neonatal mortality and marked adverse clinical signs in the offspring were observed at 2800 and 4900 ppm.

In the developmental neurotoxicity study, etofenprox technical (batch No. 87137, purity 99.2%) was administered in the diet to 24 female Sprague-Dawley (Crl:CD(SD)BR IGS) rats per group at a

concentration of 0, 250, 700 or 2100 ppm from gestation day 6 through postnatal day 21. The average dose of etofenprox was 0, 28.4, 79.2 and 238 mg/kg bw per day during gestation. Maternal clinical signs, body weights and feed consumption were recorded. A FOB was performed on 10 dams per dose on gestation days 5, 12 and 18 and on lactation days 4, 11 and 21.

All F_1 progeny were examined at 24 hours after birth, and numbers of live and dead progeny, body weights, sexes and clinical signs were recorded. Subsequently, clinical signs, mortality and body weights were recorded for all litters until rats were 28 days of age. On postnatal day 4, litters were culled to yield four males and four females (as closely as possible). Offspring representing at least 20 litters per dose were allocated for detailed clinical observations (FOB), assessment of motor activity, assessment of auditory startle response habituation, assessment of auditory startle pre-pulse inhibition, assessment of learning and memory, and neuropathology at study termination (days 63–67 of age). On postnatal day 21, the whole brain was collected from each of 10 pups of each sex per dietary level for histopathological examination and morphometric analysis. Pup sexual maturation was assessed by age at vaginal opening for females and age at completion of balano-preputial separation for males.

Randomly selected F_1 progeny (10 of each sex per group) were allocated to detect neurotoxicity using validated parameters. The preserved tissues of progeny from females treated at 0 or 2100 ppm were examined histopathologically. In addition, morphometric measurement of the thicknesses of the neocortex, corpus callosum, hippocampus and folia of the cerebellum (pyramis) was performed.

At necropsy at 63–67 days of age, brain morphometry and histopathological examination of central and peripheral nervous system tissues were performed. The brains were transected from the spinal cord above the first cervical spinal nerve, weighed, measured between the rostral part of the cerebral hemispheres and the most caudal part of the cerebellum and at the widest part of the cerebral hemispheres and then preserved. Histopathology was examined in the 0 and 2100 ppm groups. The areas of brain examined and brain morphometric measurements were as for day 21 progeny.

Parental females and their progeny not selected for neuropathology were necropsied, and a detailed postmortem examination was performed on day 21 of lactation. All examinations, including weighing, necropsy examinations and histopathological examination and measurement procedures on maternal animals and progeny, were performed without knowledge of the treatment group.

Concentrations in the diets were acceptable. There were no maternal deaths or treatment-related clinical findings at any dose level. Treatment-related maternal findings in the FOB included a consistently higher rearing activity at 2100 ppm on day 18 of gestation. Females treated at 700 or 2100 ppm showed a slight, transient decrease in weight gain from days 6 to 10 of gestation. Thereafter, weight gains were comparable to the control gain. Feed consumption was unaffected by treatment. There were no treatment-related maternal gross necropsy findings or effects on absolute and relative brain weights at any dose level.

There were no effects of treatment on the duration of gestation, littering performance, implantation number, post-implantation survival index, litter size at birth, sex ratio or offspring survival up to day 14 of lactation at any dose level. At 2100 ppm, a treatment-related marginal increase in pre-weaning mortality occurred between days 14 and 21, during which time 5.7% of progeny died, compared with 0.6% of the control progeny. However, overall pup mortality to weaning was comparable in all treated and control groups.

Although the post-weaning survival of the F_1 progeny was not changed by the treatment at any dose level, clinical signs occurred in a small number of F_1 progeny of females treated at 700 and 2100 ppm. The effects at 2100 ppm included increased incidences of dark or opaque and/or enlarged, prominent eyes and subcutaneous haemorrhagic lesions manifested as apparent cuts or reddening or bruising of the tail and/or paws, which were observed in several animals (Table 32). The effects at 700 ppm were confined to a slightly increased incidence of the eye lesion only. The incidences at

Table 32. Summary of treatment-related clinical signs in F_1 progeny in a neurodevelopmental study in rats

Clinical signs	No. of progeny (litters) affected			
	Dietary concentration (ppm)			
	0	250	700	2100
One eye large/prominent and dark	0	0	1 (1)	8 (6)
One eye large/prominent	0	1[a] (1)	1[a] (1)	1 (1)
One eye large and opaque	1 (1)	1[a] (1)	0	0
One or both eyes opaque	0	0	3[a] (1)	1 (1)
One or both eyes dark	0	0	0	2 (2)
Cut/bleeding on tail	0	0	0	2 (2)
Reddened/swollen/bruised areas on tail	1 (1)	0	3 (1)	4 (3)
Cut/bleeding on toes/paws	1	1	1 (1)	2 (2)
Swollen/bruised/redden paw(s)	1 (1)	1 (1)	1 (1)	5 (4)

From Myers (2003)

[a] First recorded post-weaning.

250 ppm were comparable to the control incidences. Further veterinary examination of six progeny at 2100 ppm and one at 700 ppm confirmed the presence of intraocular haemorrhage. These changes were considered to be treatment related. The ocular toxicity seen in this study is the outcome of the subcutaneous haemorrhage.

The body weights and subsequent weight gains of F_1 progeny of both sexes were unaffected by treatment. There was no effect of treatment at any dose level on the sexual development of F_1 progeny. There was no effect of treatment at any dose level on the behavioural development of offspring during the maternal treatment period. The auditory startle response of female offspring indicated a treatment-related influence at 2100 ppm (Table 33). The amplitude of the response was generally higher, and habituation, as measured by the decrease in amplitude of the response, was reduced in these animals at day 23/24 of age. The effect on amplitude was more marked by day 58 of age. Males at 700 and 2100 ppm also showed a shorter latency to peak response at 58 days of age, which was considered not to be toxicologically significant. Pre-pulse inhibition of the auditory startle response was unaffected by treatment at all dose levels in 23/24-day-old progeny. At 58 days of age, male progeny at 2100 ppm showed a pre-pulse inhibition, but the difference was not significant. The mean latency to peak response with pre-pulse in these animals was significantly longer than the control value.

In 58-day-old male progeny at 2100 ppm, cage floor (low beam) activity was lower, but this finding was not considered to be toxicologically significant because there were no conclusive similar effects at earlier testing intervals and no histopathological alterations in the nervous system at 2100 ppm.

There were no effects of treatment at any dose level on the observations and semiquantitative measurements made in the hand or in the standard arena on selected F_1 progeny up to 60 days of age. Similarly, there was no effect of treatment on learning or memory at any age tested at any dose level. No treatment-related gross findings were detected. The absolute and relative brain weights and brain external dimensions of all treated groups and the thicknesses of the neocortex, hippocampus, corpus callosum and cerebellum of 21-day-old progeny of both sexes at 2100 ppm were unaffected by treatment. There were no treatment-related histopathological alterations in the brains of 21-day-old progeny at 2100 ppm. Treatment-related necropsy findings in 63- to 67-day-old progeny were confined to a higher proportion of males in all treatment groups with unilateral or bilateral renal pelvic dilatation.

Table 33. Auditory startle response: peak startle amplitude, habituation and latency to peak response in a developmental neurotoxicity study in rats

Dietary concentration (ppm)	Age (days) / sex	Group mean value				
		Trial numbers				
		1–10	11–20	21–30	31–40	41–50
Startle amplitude						
0	23/24 / males	198.3	163.7	142.3	152.2	147.9
250		206.2	160.8	164.2	164.1	150.8
700		170.7	175.5	168.3	159.5	142.6
2100		192.5	186.1	177.0	168.0	153.3
0	23/24 / females	164.2	148.9	131.0	121.9	128.7
250		149.5	126.5	123.4	120.1	121.5
700		166.8	136.8	142.9	141.0	141.4
2100		186.5	181.6	174.0	168.9*	179.6
0	58 / males	383.4	317.9	311.1	294.8	292.0
250		325.7	307.6	282.9	316.1	338.2
700		403.6	330.6	316.6	311.0	310.6
2100		492.4	413.6	440.5	385.4	471.2*
0	58 / females	310.9	235.3	239.3	241.4	257.8
250		335.5	259.5	299.7	273.9	246.0
700		332.9	280.6	280.0	298.4	304.9
2100		408.4	381.8*	342.8*	352.5*	372.4**
Latency (ms)						
0	23/24 / males	19.1	19.4	23.0	21.7	23.0
250		24.2	25.3	26.4	24.7	26.6
700		23.0	23.1	23.4	24.7	24.2
2100		18.8	21.1	21.0	23.1	20.8
0	23/24 / females	22.2	22.2	20.6	21.5	23.4
250		20.5	22.2	19.6	21.7	21.7
700		22.6	21.4	22.2	22.6	23.6
2100		22.1	22.0	22.4	23.0	22.6
0	58 / males	21.1	16.4	20.2	18.4	17.6
250		20.8	17.9	13.5	15.3	13.3
700		14.8*	13.6	14.3	13.6*	15.6
2100		13.9*	15.8	15.5	12.0*	13.2
0	58 / females	18.1	16.7	17.1	15.6	16.5
250		16.0	15.5	14.2	15.7	16.7
700		16.4	16.0	15.6	16.5	16.9
2100		15.1	15.2	15.1	13.3	14.4

From Myers (2003)
* $P < 0.05$; ** $P < 0.01$

Histopathological examination of kidneys from control and 2100 ppm progeny revealed cortical tubular dilatation and hydronephrosis at similar incidences in both groups. Female progeny were not affected. The absolute and relative brain weights and brain external dimensions of all treated groups and the thicknesses of the neocortex, corpus callosum, hippocampus and cerebellum of 65-day-old progeny of both sexes at 2100 ppm were unaffected by treatment. There were no treatment-related histopathological alterations in the tissues of the central or peripheral nervous system in 65-day-old offspring from females treated at 2100 ppm.

The NOAEL for maternal toxicity was 700 ppm (equal to 79.2 mg/kg bw per day), based on increased incidence of rearing behaviour in the FOB seen at 2100 ppm (equal to 238 mg/kg bw per day). The NOAEL for offspring toxicity was 250 ppm (equal to 28.4 mg/kg bw per day), based on eye abnormalities in both sexes seen at 700 ppm (equal to 79.2 mg/kg bw per day) (Myers, 2003).

(d) Thyroid function and hepatic microsomal enzyme induction

A study was performed to investigate the effects of etofenprox on the induction of specific hepatic microsomal enzymes in the liver and their influence on the thyroid. The results were used to elucidate the mechanism of enhanced thyroid adenoma formation in a long-term study in rats (Green et al., 1986a).

Four groups of 20 male and 20 female Sprague-Dawley-derived 6- to 7-week-old rats were treated with etofenprox (batch No. 87031, purity 99%) in the diet (with 2% corn oil) at 0, 1250, 5000 or 20 000 ppm. Ten rats of each sex per group were treated for 2 weeks; five animals of each sex per group were killed at the end of the treatment period, and the remaining rats were killed after a 4-week treatment-free recovery period. In addition, 10 rats of each sex per group were treated for 4 weeks; 5 rats of each sex per group were killed at the end of the treatment period, and the remaining rats were killed after a 4-week treatment-free recovery period. The concentrations, stability and homogeneity of the formulated diets were analysed. Morbidity or mortality, clinical signs, body weights and feed consumption were recorded. Three days before sacrifice, up to five animals of each sex per group were implanted with an osmotic pump pre-loaded with 5-bromo-2′-deoxyuridine (BrdU) to detect cell proliferation activity in the liver and thyroid. After blood was collected, all animals were necropsied. Serum T_4, T_3 and TSH concentrations were measured. Liver and left thyroid/parathyroid weights were recorded. Liver, thyroid and duodenum were examined histologically at 0 or 20 000 ppm. The microsomal fraction and subsequent determination of microsomal protein concentration and the uridine diphosphate glucuronosyltransferase (UDPGT) activity using both the 4-methylumbelliferone glucuronosyltransferase (4-MUGT) method (Ullrich & Bock, 1984; Collier et al., 2000) and the *p*-nitrophenol glucuronosyltransferase (p-NPGT) method (Winsnes, 1969; Burchell & Coughtrie, 1989) were measured. The right thyroid was analysed for thyroid microsomal peroxidase (TP) activity (Peterson, 1979). BrdU incorporated into S-phase cells was localized by a chromagen and counter-stained with haematoxylin. The slides were first examined under low magnification, and BrdU labelling was quantified under high power by examination of at least 3500 hepatocytes in 10 random fields per animal and 500 thyroid follicular cells per animal. The stability, homogeneity and concentrations of the diets were acceptable. Five animals including controls died during the osmotic pump implantation procedure. No treatment-related clinical signs were evident. Body weight gain in both sexes at 20 000 ppm was reduced at 14 and 28 days of treatment. The body weights in both groups of recovery animals were comparable to those of controls. The results are shown in Table 34.

No treatment-related changes in serum T_3 activity were detected by 2 or 4 weeks of treatment. Serum T_4 concentration was significantly lower at 20 000 ppm after 2 weeks of treatment. The decreases in T_4 values in males were fully reversible during the treatment-free period. Treatment-related effects on microsomal protein yield were increased in males treated for 4 weeks at 20 000 ppm, and the effect was reversible. Hepatic microsomal 4-MUGT activity was increased in both sexes treated for 2 weeks at 20 000 ppm and in males at 5000 ppm. The effects showed some degree of reversibility, but enzyme activities remained significantly ($P < 0.05$ or 0.01) higher after a 2-week treatment-free period. There was

Table 34. Summary of investigative study on thyroid function and hepatic microsomal enzyme induction in rats treated with etofenprox

No. of weeks of treatment ± recovery period of 4 weeks	Group mean values							
	Males				Females			
	Dietary concentration (ppm)							
	0	1250	5000	20 000	0	1250	5000	20 000
TSH concentration (ng/ml)								
2 weeks / without recovery	0.123	0.320[a]	0.318	0.537*	0.057[b]	0.116[b]	0.177	0.354*
4 weeks / without recovery	0.218	0.551	0.346	0.53	0.063	0.13	0.202*	0.369*
2 weeks / with recovery	0.436	0.24	0.285	0.124	0.105	0.147	0.123	0.106
4 weeks / with recovery	0.306	0.424	0.27	0.211	0.093	0.098	0.094	0.09
T_3 concentration (ng/dl)								
2 weeks / without recovery	92.16	83.87[a]	82.82	92.80[b]	98.25[b]	112.58[b]	104.94	102.55[b]
4 weeks / without recovery	92.04	92.68	110.02	99.24	105.94	114.02	105.36	116.3
2 weeks / with recovery	72.16	88.94	94.8	86.04	87.34	76.66	89.28	83
4 weeks / with recovery	72.02	83.84	85.46	79.52	103.92	90	98.02	97.12
T_4 concentration (mg/dl)								
2 weeks / without recovery	6.1	5.3[a]	4.8	4.0*	3.4[b]	4.6[b]	3.7	3.0[b]
4 weeks / without recovery	6	5.8	5.6	4.6	3.6	4.6	3.9	3.6
2 weeks / with recovery	3.5	3.4	4.3	4.3	2.6	2.7	3	2.8
4 weeks / with recovery	4.4	5.2	5.2	4.9	3.2	2.8	3	3.9
Microsomal protein (mg/g liver)								
2 weeks / without recovery	45.1	49.7[a]	49.2	51.8[b]	30.8[b]	32.4[b]	32.7	36.3
4 weeks / without recovery	44.5	45.7	47.8	59.9**	36.4	37.2	39	37
2 weeks / with recovery	39.7	38.3	47.8*	46	30.7	36.3	34.3	42.4*
4 weeks / with recovery	39.3	38.6	37.2	40.7	43.6	47.6	47	49.9
4-MUGT activity (nmol/mg protein per minute)								
2 weeks / without recovery	9.94	9.99[a]	13.5**	18.0**[b]	12.3[b]	13.1[b]	14.1	19.0**
4 weeks / without recovery	7.42	7.15	8.72	7.14	12.1	13.9	12.1	14.7
2 weeks / with recovery	9.73	9.63	12.2*	15.9**	9.97	9.91	12.4*	14.5**
4 weeks / with recovery	8.76	8.94	9.74	10.7	9.33	9.74	9.41	10.4
***p*-NPGT activity (nmol/mg protein per minute)**								
2 weeks / without recovery	13.9	12.6[a]	18.6	26.7**[b]	12.9[b]	17.7[b]	19.8	32.5**
4 weeks / without recovery	21.8	20.7	19	19.4	13.1	14	13.6	15.8
2 weeks / with recovery	18.6	24.2	26.6*	34.7**	12.2	13.3	17.8	24.5**
4 weeks / with recovery	13.5	16.2	15.6	20.3	12.3	11.1	9.8	13.3
TP activity (optical density/mg protein per minute)								
2 weeks / without recovery	1.04	0.84	1.1	1.05	1.01	0.83	1.12	1.71
4 weeks / without recovery	1.25	0.78	0.79	0.2	1.25	0.75	0.74	0.88
2 weeks / with recovery	1.27	0.64	0.85	0.9	1.01	0.73	0.92	0.98
4 weeks / with recovery	0.99	0.44	0.82	0.68	0.35	0.85	0.95	0.76

From Smith (2003a)

* $P < 0.05$; ** $P < 0.01$

[a] Mean of three animals only, excluded from statistical analysis.

[b] Mean of four animals.

no apparent effect (non-significant) on hepatic microsomal 4-MUGT activity after 4 weeks of treatment, including the recovery period. Hepatic microsomal UDPGT activity was significantly increased in both sexes treated for 2 weeks at 20 000 ppm and showed a higher trend in both sexes at 5000 ppm. The increased activities remained after the recovery period at 5000 and 20 000 ppm. TP activity was higher in females for 2 weeks at 20 000 ppm compared with controls, but was not statistically significant. All treated groups of both sexes showed lower TP activity compared with controls after 4 weeks of treatment, and TP activity remained slightly depressed in males after the 4-week treatment-free period. Hepatic labelling indices were comparable to control values in all groups and in both sexes. The thyroid labelling indices in males treated at 20 000 ppm for 2 or 4 weeks were greater than control values but not significantly, and those in females were decreased, suggesting no increased cell proliferation in the thyroid for 4-week treatment. No treatment-related gross lesions in any of the treated groups were observed. There was a dose-related increase in the absolute and relative liver weights of males for 2 or 4 weeks at 5000 or 20 000 ppm, and the increase showed a substantial degree of recovery. The absolute and relative thyroid weights of both sexes for 2 or 4 weeks showed a higher trend at 20 000 ppm, but none of the values were statistically significant. The increases in thyroid weights at 1250 and 5000 ppm were considered to be probably treatment related, because of the consistency and magnitude of the differences. Microscopically multinucleated hepatocytes after 2 and 4 weeks of treatment, which persisted to the end of the treatment-free periods, and centrilobular hepatocyte hypertrophy in both sexes after 2 weeks and in females only after 4 weeks were detected.

Hepatic microsomal enzyme induction (UDPGT) by the chronic administration of xenobiotic chemicals leading to thyroid follicular cell neoplasms is a well-known pathway in rodents (McClain et al., 1988; Capen, 2007). Long-term exposure of rats to a wide variety of different chemicals may induce UDPGT pathways and result in chronic stimulation of the thyroid by increased TSH levels. The chronic stimulation of the thyroid by increased TSH activity often results in a greater risk of developing thyroid follicular cell tumours in rats. In addition, T_4 metabolism takes place very rapidly in rats because of the absence of T_4 binding globulin in this species (McClain et al., 1988; Capen, 2007). This tumour development pathway is rodent specific and not relevant to humans.

Although the increase in TSH activity and/or decrease in T_4 levels were not consistent in 4 weeks of treatment with etofenprox, UDPGT induction in the liver was clear. The toxicological data obtained indicate that etofenprox has a similar pathway to that described in the above paragraph, and a mode of action (MOA) analysis is provided in Appendix 1.

The NOAEL for the primary effect on the liver was 1250 ppm (equal to 81.2 mg/kg bw per day), based on the occurrence of increased hepatic UDPGT activity at 5000 ppm (Smith, 2003a).

(e) Immunotoxicity

Mice

In an immunotoxicity study, etofenprox (lot No. K0561075, purity 98.4%) was administered to 10 Crl:CD-1 (ICR) mice of each sex per dose in the diet at a dose level of 0, 320, 1600 or 8000 ppm (equal to 0, 50, 239 and 1116 mg/kg bw per day in males and 0, 60, 284 and 1528 mg/kg bw per day in females, respectively). The positive control group, consisting of eight Crl:CD-1 (ICR) mice of each sex, was administered five daily oral gavage doses of cyclophosphamide at 20 mg/kg bw between days 22 and 26. Clinical observations, body weight, body weight gain, feed consumption, water consumption and organ weight were evaluated. On day 25, all test animals were immunized with a 0.2 ml intravenous injection of sheep red blood cells in 0.9% saline. All animals were necropsied after 4 weeks (on day 29) of treatment, and the weights of spleen and thymus were recorded. The spleens from all test, control and positive control group mice were used as a source of splenocytes for assessment of the adaptive or acquired immune response to the T cell–dependent immunogen, sheep red blood cells, using a modification of the Jerne plaque-forming cell (PFC) assay. The number

of lytic plaques for each animal was determined, and group mean responses were expressed as group mean number of PFCs per spleen and per 10^6 viable splenocytes.

All mice survived the scheduled treatment period, and there were no treatment-related clinical signs. Mice of both sexes at 8000 ppm showed loss of body weight during the first 4 days of the treatment period, and overall body weight gains of males and females were 84% and 47% lower than those of controls, respectively. The dose level of 8000 ppm was considered to exceed the maximum tolerated dose for a study of this duration. The body weights of males and females receiving 320 or 1600 ppm were not affected by treatment. The overall feed consumption of the male group treated at 8000 ppm was reduced by 11% relative to the controls, and water consumption of both sexes at this dose was increased up to 71.4% compared with controls.

Spleen and thymus weights were unaffected by treatment at all dose levels. At necropsy, enlarged liver was observed in some animals of both sexes at 1600 and 8000 ppm. There was no effect of treatment in either sex at any dose level on the T cell–dependent humoral antibody response to injected sheep red blood cell antigen, as assessed by the modified Jerne PFC assay. In contrast, treatment with cyclophosphamide at 20 mg/kg bw per day resulted in a significant decrease of the PFC response to a challenge with sheep red blood cell antigen, demonstrating the sensitivity of the test system to a known immunosuppressant. These results indicated that etofenprox administered to male and female CD-1 mice at dose levels up to 1116 mg/kg bw per day in males and 1528 mg/kg bw per day in females for 4 weeks did not affect the T cell–dependent humoral antibody response to injected sheep red blood cell antigen, as assessed by a modified Jerne PFC assay.

The NOAEL for immunotoxicity in mice was 1116 mg/kg bw per day, the highest dose tested. The NOAEL for all effects was 239 mg/kg bw per day, based on the occurrence of reduced body weight gain and feed consumption at 1116 mg/kg bw per day. Etofenprox has no immunotoxicity potential in mice (Moore, 2010).

Rats

In an immunotoxicity study, etofenprox (lot No. K0561075, purity 98.4%) was administered to 10 male Crl:CD(SD)IGS BR rats per dose in the diet at a dose level of 0, 560, 2800 or 14 000 ppm (0, 44, 213 or 1053 mg/kg bw per day, respectively). The positive control group consisted of eight male Crl:CD(SD)IGS BR rats administered a single 50 mg/kg bw intraperitoneal bolus injection of cyclophosphamide on day 27. All rats received a sensitizing intravenous dose of sheep red blood cells in 0.9% saline 4 days prior to termination. Clinical observations, body weight, body weight gain, feed consumption, water consumption and organ weights were evaluated. All animals were necropsied after 4 weeks of treatment, and the weights of spleen and thymus were recorded. The spleen from all tests, controls and positive control animals was used as a source of splenocytes for assessment of the adaptive or acquired immune response to the T cell–dependent immunogen, sheep red blood cells, using a modification of the Jerne PFC assay. The number of lytic plaques for each animal was determined, and group mean responses were expressed as group mean number of PFCs per spleen and per 10^6 viable splenocytes.

All rats survived the scheduled treatment period, and there were no treatment-related clinical signs. Reduced body weight gain and feed consumption occurred at 1053 mg/kg bw per day. Water intake was unaffected by treatment at all dose levels. There were no treatment-related gross lesions at necropsy, and both unadjusted and adjusted spleen and thymus weights were unaffected by treatment at all dose levels. There were no statistically significant differences in the numbers of cells per spleen, PFCs per 10^6 viable cells or PFCs per spleen in any of the etofenprox-treated groups when compared with the vehicle control group. In the cyclophosphamide-treated group, the numbers of cells per spleen, PFCs per 10^6 viable cells and PFCs per spleen were all statistically significantly reduced to a marked extent, demonstrating the sensitivity of the test system to a known immunosuppressant. These results indicated that dietary administration of etofenprox to male Sprague-Dawley rats at dose

levels up to 1053 mg/kg bw per day for 4 weeks did not affect the T cell–dependent humoral antibody response to injected sheep red blood cell antigen, as assessed by a modified Jerne PFC assay.

The NOAEL for immunotoxicity was 14 000 ppm (equal to 1053 mg/kg bw per day), the highest dose tested. The NOAEL for systemic toxicity was 1600 ppm (equal to 213 mg/kg bw per day), based on the occurrence of reduced body weight gain and feed consumption at 8000 ppm (equal to 1053 mg/kg bw per day). Etofenprox has no immunotoxicity potential in rats (Groom et al., 2010).

(f) Toxicity of metabolites

Oral and dermal acute toxicity studies, a 13-week toxicity study and genotoxicity studies of a plant metabolite of etofenprox, α-CO, were conducted.

Acute oral toxicity

A group of fasted young adult Sprague-Dawley rats (five of each sex) was treated orally, by gavage, with α-CO (batch No. OFU-1021, purity 99.6%) suspended in corn oil as a single dose of 5000 mg/kg bw. Animals were observed for mortality and clinical signs several times for the 1st day and twice daily thereafter for 14 days. Body weights were recorded on days 1, 8 and 15. A gross necropsy was performed on all animals.

No deaths occurred during the study. The treatment-related clinical signs were confined to decreased motor activity in all animals within 2 hours post-treatment. All animals had recovered within 4 hours after treatment. All animals gained weight throughout the observation period. There were no treatment-related gross findings at necropsy in any animal. Under the study conditions utilized, the acute oral LD_{50} value is greater than 5000 mg/kg bw (Cummins & Gardner, 1985a).

Acute dermal toxicity

A group of five male and five female young adult Sprague-Dawley rats was treated with α-CO (batch No. OFU-1021, purity 99.6%) dispersed in water for 24 hours by topical, occluded application to a clipped area of intact dorsal skin at a dose level of 2000 mg/kg bw. After 24 hours of exposure to the test substance, aluminium foil and waterproof bandages were removed, and the application sites were wiped with wetted paper towels. Animals were observed for mortality and clinical signs several times for the 1st day and once daily thereafter during the 14-day observation period. Body weights were recorded pre-dosing and on days 1, 8 and 15. The animals were subjected to necropsy and postmortem examination.

No deaths or systemic clinical signs of an adverse reaction to treatment occurred. There were no local signs of an effect of treatment at the application site, and there were no macroscopic findings at necropsy in any animal. All animals gained weight during the study. Based on these results, the acute dermal LD_{50} was estimated to be greater than 2000 mg/kg bw (Cummins & Gardner, 1985b).

Subchronic toxicity

In a 4-week toxicity study, four groups of five male and five female 5- to 6-week-old Sprague-Dawley-derived rats (Crl:CD (SD)) were treated orally for 4 weeks with α-CO (batch No. ST 401, purity 97.6%) at a dietary concentration of 0, 1500, 5000 or 15 000 ppm (equal to 0, 156, 525 and 1718 mg/kg bw per day in males and 0, 162, 543 and 1643 mg/kg bw per day in females, respectively). Morbidity/mortality checks were performed twice daily, and detailed clinical examinations were performed daily. Body weights and feed consumption were recorded weekly throughout the study. All animals were subjected to necropsy after 4 weeks of treatment. No histopathological examinations were performed.

There were no deaths during the study. There were no treatment-related clinical signs. There was a treatment-related, statistically significant reduction in body weight gain in both sexes at 15 000 ppm. The overall weight gain was reduced by 29.5% and 53.9% in males and females, respectively. Feed and water consumption were not affected by the treatment. There were no treatment-related findings

at necropsy at any dose level. Liver and kidney weights were increased in males at 15 000 ppm. Male kidney weights at 5000 ppm were also significantly increased. The effects on organ weights were not apparent at 1500 ppm in males or in any of the female treated groups. All other group mean organ weight values were not significantly different ($P > 0.05$) from control values.

The NOAEL was 1500 ppm (equal to 156 mg/kg bw per day), based on increased kidney weights in males seen at 5000 ppm (equal to 525 mg/kg bw per day) (Powell et al., 1987).

In a 90-day toxicity study, four groups of 10 male and 10 female 6- to 7-week-old Sprague-Dawley-derived rats (Crl:CD(SD)BR) were treated orally for 13 weeks with α-CO (batch No. ST401, purity 97.6%) at a dietary concentration of 0, 50, 700 or 10 000 ppm (equal to 0, 3.8, 54 and 805 mg/kg bw per day in males and 0, 4.7, 64 and 932 mg/kg bw per day in females, respectively). The stability and homogeneity of the 50 and 10 000 ppm diets were confirmed prior to the start of the study. Morbidity/mortality checks were performed twice daily, and detailed clinical examinations were performed daily. Body weights, feed consumption and water consumption were recorded periodically. Ophthalmoscopic examinations were performed on all animals pre-dosing and on all animals treated at 0 and 10 000 ppm at weeks 6 and 13. Haematology (including thrombotest), blood chemistry (including T_3 and T_4 activities) and urine analyses were performed on all animals during week 13. All animals were subjected to necropsy after 13 weeks of treatment. Selected organs were weighed and preserved. Sections of all tissues, except femur and the head, from the animals treated at 0 and 10 000 ppm were examined by light microscopy. In addition, the lungs, liver, kidneys and gross lesions from all animals were examined histologically.

The dietary concentrations, homogeneity and stability were acceptable. There were no deaths or treatment-related clinical signs at any dose level during the study. The body weight gain was reduced throughout the study in both sexes treated at 10 000 ppm. Body weight gains during weeks 1–12 were statistically significantly reduced by 15% and 17% in males and females treated at 10 000 ppm, respectively. Feed and water consumption were not affected by the treatment. No treatment-related ocular lesions were observed in the animals treated at 10 000 ppm at weeks 6 and 13. There were no treatment-related effects on the haematological profile of either sex at any dose level after 13 weeks of treatment. Plasma alkaline phosphatase activity in both sexes and AST activity in males were statistically significantly increased at 10 000 ppm. Circulating T_4 concentrations were statistically significantly reduced in males and females at 10 000 ppm by 52.9% and 23.8% relative to the controls, respectively. The effect in males was accompanied by a significantly ($P < 0.01$) reduced circulating T_3 concentration. In females at all dose levels, T_3 concentrations were statistically significantly elevated by up to 27.5%. T_4 and T_3 concentrations in animals treated at lower dose levels were not affected. A dose-related decrease in total protein and globulin concentrations occurred in both sexes at 10 000 ppm and in males at 50 and 700 ppm. In males at 10 000 ppm, urine volume was decreased, which resulted in increased specific gravity. There were no treatment-related gross lesions at necropsy at any dose level. Females treated at 10 000 ppm showed statistically significantly increased adjusted and relative liver and kidney weights, whereas relative kidney weights were statistically significantly increased in males treated at 10 000 ppm. Treatment-related histopathological findings were confined to 4 of 10 females treated at 10 000 ppm that showed hypertrophy of the renal tubular epithelium of the pars recta. As the change was not associated with evidence of renal functional deficit, degenerative alterations or hyperplasia, the histological alteration was considered to be of little toxicological relevance. There were no other treatment-related histopathological alterations in any tissue.

The NOAEL was 700 ppm (equal to 54 mg/kg bw per day), based on reduced weight gain, increased plasma alkaline phosphatase and AST activities, reduced circulating T_4 and total protein concentrations, increased liver and kidney weights and minor histopathological alterations in the kidneys (females) at 10 000 ppm (equal to 805 mg/kg bw per day) (Powell et al., 1988).

Mutagenicity

α-CO (batch No. OFU-1021, purity 99.6%) was tested for mutagenicity in *Salmonella typhimurium* and *Escherichia coli*, a DNA damage assay in *E. coli* and an in vitro clastogenicity assay using human lymphocytes. No significant responses were observed in these assays in the presence or absence of metabolic activation. Based on the results of these assays, α-CO is not likely to be genotoxic (Bootman & May, 1985a,b; Bootman, Hodson-Walker & Dance, 1985c).

3. Observations in humans

There are no clinical cases or poisoning incidents relating to etofenprox that are known to the registrant.

Comments

Biochemical aspects

In rats given a single oral dose of 1:1 [^{14}C]etofenprox mixture labelled on either side of the ether linkage, absorption was rapid but incomplete, to the extent of approximately 64–68% of the dose at 30 mg/kg bw and 48–58% of the dose at 180 mg/kg bw. The time to reach maximum concentrations in plasma was 2–7 hours. Distribution to the tissues was extensive after seven daily doses of 30 mg/kg bw, with tissue concentrations reaching their maxima 4 hours after the last dose. Highest concentrations were found in fat, adrenals, liver, ovaries and thyroid. Apart from the gastrointestinal tract, which contained much unabsorbed material, concentrations elsewhere, including brain, were low. Etofenprox crossed the placenta to the fetus, but placental and fetal concentrations were low relative to maternal plasma concentrations, and elimination from the placenta and the fetus was rapid. Unmetabolized etofenprox was secreted into maternal milk. Depletion from the tissues was rapid except from fat, in which estimated half-lives were approximately 5 and 8.5 days in males and females, respectively. In rats with bile duct cannulae, radiolabelled etofenprox administered at 30 mg/kg bw to males and 180 mg/kg bw to males and females was rapidly eliminated, with almost 90% combined excreted in the bile (10–15%) and faeces (75–78%) and approximately 1–3% in the urine within 48 hours; females receiving 30 mg/kg bw eliminated the radioactivity differently, with 30%, 50% and 3% appearing in bile, faeces and urine, respectively, in 0–48 hours. This difference was not observed in rats without cannulae. The routes and extent of elimination of etofenprox and its metabolites were independent of the dose level and the sex of the rats. No unchanged etofenprox or a key primary metabolite, α-CO, has been recovered from urine.

In dogs given the 1:1 [^{14}C]etofenprox mixture orally, the rate of absorption of radioactivity was quite variable, with maximum plasma concentrations occurring 0.25–6 hours after dosing. The extent of absorption was approximately 40–50%. This was followed by approximately 90% faecal elimination (excretion and non-absorbed etofenprox combined) and 10% urinary excretion, almost all occurring within 24 hours. Very high concentrations were found in bile, none of which was due to parent etofenprox.

In rats, no unchanged etofenprox was found in urine, whereas in faeces, it was one of the major components, most likely due to unabsorbed material. Cleavage of the etofenprox molecule did not appear to be a significant metabolic process, although a significant number of radiolabelled entities were not identified. In faeces, desethyletofenprox occurred at 19.5–25.1% of the dose, and etofenprox hydroxylated in the 4′ position of the phenoxybenzyl moiety occurred at 7.2–13.8% of the dose. Other primary metabolic steps involved oxidation of carbons on either side of the ether linkage, one product of which, α-CO, is a major metabolite or degradation product isolated during plant and soil and photodegradation studies. These carbonyls appear to be rapidly metabolized to scission products, some of which (m-PB-acid, m-PB-alc and 4′-OH-PB) are shared with other pesticides. Glucuronide and sulfate conjugates were also found.

Toxicological data

The acute oral and dermal LD_{50} values in rats are both greater than 2000 mg/kg bw. The acute oral LD_{50} value in the dog is greater than 5000 mg/kg bw. The acute 4-hour inhalation LC_{50} value in the rat is greater than 5.88 mg/l. Etofenprox was not irritating to rabbit skin or rabbit eyes. Etofenprox was not a skin sensitizer in the guinea-pig maximization test.

The liver is a common target for the toxicity of etofenprox in mouse, rat and dog. The liver, kidneys and haemolymphoreticular system were identified as target organs in the mouse. The NOAEL in a 90-day toxicity study in mice was 3000 ppm (equal to 375 mg/kg bw per day), based on increased mortality and the occurrence of reduced body weight gain and feed consumption, increased water consumption, minor haematological effects, histopathological alterations indicative of kidney damage and minor changes in liver and the lymphoreticular system at 15 000 ppm (equal to 1975 mg/kg bw per day).

The liver and thyroid gland were the target organs in the rat. In a 90-day toxicity study in rats, the NOAEL was 300 ppm (equal to 20 mg/kg bw per day), based on liver toxicity (hepatocyte enlargement and clinical evidence of liver dysfunction affecting fat metabolism and the synthesis of blood clotting factors) and thyroid toxicity (an increase in the number of thyroid microfollicles and reduced levels of circulating T_4) at 1800 ppm (equal to 120 mg/kg bw per day).

The NOAEL in a 1-year dog study was 1000 ppm (equal to 32.2 mg/kg bw per day), based on hepatotoxicity, including increased liver weights in both sexes and histopathological alterations in females at 10 000 ppm (equal to 339 mg/kg bw per day). The hepatic effects were reversible.

The carcinogenic potential of etofenprox was studied in mice and rats. In the 2-year toxicity and carcinogenicity study in mice, the NOAEL for non-neoplastic effects was 30 ppm (equal to 3.1 mg/kg bw per day), based on an increased incidence of dilated/basophilic renal cortical tubules at 100 ppm (equal to 10.4 mg/kg bw per day). At higher doses, the renal lesions were characterized as an increased incidence of cortical scarring and pale coloration, organ enlargement, dilated or cystic Bowman's capsules, dilated medullary tubules, focal loss of tubules, prominent interstitial papillary tissue and papillary mineralization. There were also small increases in reticulum cell sarcomas in female mice at 100 ppm and above. These reticulum cell sarcomas were not considered treatment related because of a lack of a dose–response relationship, and they are common tumours in mice. The combined incidence of renal cortical adenomas and carcinomas was marginally non-statistically significantly increased in males at 700 and 4900 ppm. Nevertheless, these tumours are rare in mice and were slightly above the historical control range. Therefore, they were considered treatment-related tumours. It is plausible that the continuous stimulation by chronic renal toxicity was responsible for renal tumour development. The NOAEL for carcinogenicity in mice was 100 ppm (equal to 10.4 mg/kg bw per day), based on renal cortical tumours at 700 ppm (equal to 75.2 mg/kg bw per day).

In the chronic toxicity and carcinogenicity study in rats, the NOAEL for non-neoplastic effects was 100 ppm (equal to 3.7 mg/kg bw per day), based on an increase in foci or areas of eosinophilic hepatocytes in males and vacuolated hepatocytes in females and reduced body weight gain in males at 700 ppm (equal to 25.5 mg/kg bw per day). The thyroid follicular cell adenomas and carcinomas combined were statistically significantly increased in females at 4900 ppm. Increased thyroid follicular cell adenomas and carcinomas combined were also observed in males at 4900 ppm, but statistical significance was not achieved. The NOAEL for carcinogenic effects in female rats was 700 ppm (equal to 34.3 mg/kg bw per day), based on an increased incidence of thyroid follicular cell adenomas and carcinomas combined at 4900 ppm (equal to 249.1 mg/kg bw per day).

A mechanistic study in rats was conducted to clarify the relationship between a primary effect of etofenprox on hepatic microsomal enzyme induction and thyroid follicular cell adenoma development. Etofenprox increased UDPGT activity in the liver, which would be expected to increase excretion of T_4 from the blood. Decreased serum T_4 levels were observed with a consequent increase in TSH activity, which would be expected to result in follicular cell hyperplasia and, if sustained, tumour

development. Rodents are particularly sensitive to the induction of thyroid follicular cell tumours, firstly because of the easy induction of UDPGT and secondly because of rapid T_4 metabolism in the absence of a specific T_4 binding globulin. In other species, this provides a buffering capacity that better controls the dynamic equilibrium of hormones in the pituitary–hypothalamic–thyroid axis.

Based on MOA analysis for thyroid follicular tumours, the Meeting concluded that these tumours were not relevant for human risk assessment.

The potential genotoxicity of etofenprox was tested in an adequate range of in vitro and in vivo genotoxicity studies. No evidence of genotoxic potential was found.

The Meeting concluded that etofenprox was unlikely to be genotoxic.

On the basis of the absence of genotoxicity, the absence of carcinogenicity in rats by an MOA relevant to humans and carcinogenicity in mice likely to be secondary to renal toxicity at exposure levels of unlikely human relevance, the Meeting concluded that etofenprox is unlikely to pose a carcinogenic risk to humans.

No reproductive toxicity was observed in two multigeneration reproduction studies in rats at doses up to 4900 ppm (equal to 246 mg/kg bw per day) when administered through the diet and 5000 mg/kg bw per day when administered by gavage. The NOAEL for parental toxicity was 700 ppm (equal to 37 mg/kg bw per day), based on the occurrence of reduced weight gain, increased kidney, liver and thyroid weights and histopathological findings in the liver, kidneys and thyroid at 4900 ppm (equal to 246 mg/kg bw per day). The NOAEL for offspring toxicity was 700 ppm (equal to 37 mg/kg bw per day), based on the occurrence of increased kidney weights in females in the F_{2b} generation at 4900 ppm (equal to 246 mg/kg bw per day).

In a modified developmental toxicity study, rats were administered etofenprox by gavage during gestation days 6–17. The maternal toxicity NOAEL in rats was 250 mg/kg bw per day, based on decreased body weight gain and clinical signs at 5000 mg/kg bw per day. The developmental NOAEL was 5000 mg/kg bw per day, the highest dose tested.

In two developmental toxicity studies conducted in rabbits, the overall NOAEL for developmental toxicity and maternal toxicity was 100 mg/kg bw per day, based on the occurrence of reduced maternal body weight gain and feed consumption on gestation day 6 (first day of dosing), mortality and increased post-implantation loss and intrauterine growth retardation at the high dose of 250 mg/kg bw per day.

The Meeting concluded that etofenprox is not teratogenic in rats or rabbits.

No evidence of neurotoxicity was observed in an acute neurotoxicity study in rats at doses up to 2000 mg/kg bw. No evidence of systemic toxicity, including neurotoxicity, was observed in a 13-week neurotoxicity study in rats at doses up to 10 000 ppm (equal to 604 mg/kg bw per day). In a developmental neurotoxicity study in rats, the NOAEL for maternal toxicity was 700 ppm (equal to 79.2 mg/kg bw per day), based on an increased incidence of rearing behaviour in the FOB at 2100 ppm (equal to 238 mg/kg bw per day). The NOAEL for offspring toxicity was 250 ppm (equal to 28.4 mg/kg bw per day), based on eye abnormalities (increased incidences of dark or opaque and/or enlarged, prominent eyes and a subcutaneous haemorrhagic lesion) in both sexes seen at 700 ppm (equal to 79.2 mg/kg bw per day). The ocular toxicity seen in these studies is the outcome of the subcutaneous haemorrhage. There was no evidence of neurotoxicity in the offspring.

In immunotoxicity studies in mice and rats, no evidence of immunotoxicity was observed at doses up to 1116 and 1053 mg/kg bw per day in mice and rats, respectively.

Toxicological data on metabolite

Toxicity studies of a plant metabolite of etofenprox, α-CO, were conducted. α-CO has low acute oral and dermal toxicity in the rat. The LD_{50} values are greater than 5000 mg/kg bw and greater than 2000 mg/kg bw for oral and dermal toxicity, respectively.

The lowest NOAEL for α-CO in 4-week and 13-week dietary studies in rats was 54 mg/kg bw per day, based on the effects on the liver, kidney and thyroid in a 13-week dietary study at 10 000 ppm

(equal to 805 mg/kg bw per day). The toxicological profile of α-CO is similar to that of etofenprox, but its toxicity is lower than that of the parent (20 mg/kg bw per day). α-CO is not genotoxic.

There were no reports of adverse health effects in manufacturing plant personnel. Also, there were no reports of poisoning with etofenprox.

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

Toxicological evaluation

The Meeting confirmed the ADI of 0–0.03 mg/kg bw on the basis of the NOAEL of 3.1 mg/kg bw per day from the 108-week carcinogenicity study in mice based on renal toxicity (an increased incidence of dilated and basophilic renal tubules) at 10.4 mg/kg bw per day and using a safety factor of 100. The ADI was supported by the NOAEL of 3.7 mg/kg bw per day from the 2-year toxicity and carcinogenicity study in rats, based on an increase in foci or areas of eosinophilic hepatocytes in males and vacuolated hepatocytes in females and reduced body weight gain in males at 25.5 mg/kg bw per day. This ADI is adequately protective of renal cortical tumours occurring at higher doses in mice.

The Meeting established an acute reference dose (ARfD) of 1 mg/kg bw on the basis of the overall NOAEL of 100 mg/kg bw per day from the two developmental toxicity studies in rabbits, based on the occurrence of reduced maternal body weight gain and feed consumption during the early dosing period (gestation day 6) and increased post-implantation loss, which could occur after a single exposure, and using a safety factor of 100.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year study of toxicity and carcinogenicity[a]	Toxicity	30 ppm, equal to 3.1 mg/kg bw per day	100 ppm, equal to 10.4 mg/kg bw per day
		Carcinogenicity	100 ppm, equal to 10.4 mg/kg bw per day	700 ppm, equal to 75.2 mg/kg bw per day
Rat	Two-year study of toxicity and carcinogenicity[a]	Toxicity	100 ppm, equal to 3.7 mg/kg bw per day	700 ppm, equal to 25.5 mg/kg bw per day
		Carcinogenicity	700 ppm, equal to 34.3 mg/kg bw per day (females)	4900 ppm, equal to 249.1 mg/kg bw per day (females)
	Multigeneration study of reproductive toxicity[a]	Parental toxicity	700 ppm, equal to 37 mg/kg bw per day	4900 ppm, equal to 246 mg/kg bw per day
		Offspring toxicity	700 ppm, equal to 37 mg/kg bw per day	4900 ppm, equal to 246 mg/kg bw per day
		Reproductive toxicity	4900 ppm, equal to 246 mg/kg bw per day[b]	—
	Developmental toxicity study[c]	Maternal toxicity	250 mg/kg bw per day	5000 mg/kg bw per day
		Embryo and fetal toxicity	5000 mg/kg bw per day[b]	—
Rabbit	Developmental toxicity studies[c,d]	Maternal toxicity	100 mg/kg bw per day	250 mg/kg bw day
		Embryo and fetal toxicity	100 mg/kg bw per day	250 mg/kg bw day
Dog	One-year studies of toxicity[a,d]	Toxicity	1000 ppm, equal to 32.2 mg/kg bw per day	10 000 ppm, equal to 339 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

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 etofenprox

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid but incomplete, ~50%
Dermal absorption	Not available
Distribution	Distributed throughout the body; highest concentrations in fat, adrenals, liver, ovaries and thyroid
Potential for accumulation	None
Rate and extent of excretion	Rapid and extensive
Metabolism in animals	Desethyletofenprox and hydroxylated etofenprox
Toxicologically significant compounds (animals, plants and the environment)	Parent
Acute toxicity	
Rat, LD_{50}, oral	> 2000 mg/kg bw
Rat, LD_{50}, dermal	> 2000 mg/kg bw
Rat, LC_{50}, inhalation	> 5.88 mg/l
Rabbit, skin irritation	Non-irritating
Rabbit, eye irritation	Non-irritating
Guinea-pig, skin sensitization (maximization test)	Not a sensitizer
Short-term studies of toxicity	
Target/critical effect	Liver, reduced body weight
Lowest relevant oral NOAEL	20 mg/kg bw per day (13-week toxicity study in rats)
Lowest relevant dermal NOAEL	1000 mg/kg bw per day (rabbits, highest dose tested)
Lowest relevant inhalation NOAEC	0.21 mg/l (13-week inhalation study in rats)
Genotoxicity	
	Not genotoxic
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Kidney, liver, haematology, body weights
Lowest relevant NOAEL	3.1 mg/kg bw per day (carcinogenicity study in mice)
Carcinogenicity	Unlikely to pose carcinogenic risk to humans at dietary exposure levels
Reproductive toxicity	
Reproduction target/critical effect	Kidney/increased kidney weight

Lowest relevant reproductive NOAEL	246 mg/kg bw per day, the highest dose tested (multigeneration study in rats)
Developmental target/critical effect	Abortions and post-implantation loss
Lowest relevant developmental NOAEL	100 mg/kg bw per day (rabbits)
Neurotoxicity/delayed neurotoxicity	
Acute neurotoxicity	Not neurotoxic (rats)
Subacute neurotoxicity	Not neurotoxic (13-week study in rats)
Neurodevelopmental toxicity	Not neurodevelopmental toxicant (rats)
Immunotoxicity studies	
	Not immunotoxic (rats and mice)
Medical data	
	No adverse effects have been reported
Mechanistic studies	
	Studies on the thyroid axis that demonstrate a tumour MOA not relevant to humans

Summary

	Value	Study	Safety factor
ADI	0–0.03 mg/kg bw	Two-year carcinogenicity study in mice, supported by the 2-year toxicity and carcinogenicity study in rats	100
ARfD	1 mg/kg bw	Developmental studies in rabbits	100

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Appendix 1: Application of the IPCS conceptual framework for cancer risk assessment

A1.1 Introduction

The data relating to the MOA below are summarized in section 2.6(d). The analytical approach applied to the postulated MOA is based on the International Programme on Chemical Safety (IPCS) conceptual framework for evaluating an MOA for chemical carcinogens (Sonich-Mullin et al., 2001). Administration of etofenprox was associated with statistically significant increases in follicular cell adenomas and combined adenomas and carcinomas in the thyroid in females at 4900 ppm, the highest dose. In male rats at the same dose, the incidences were higher than in the controls, but the difference was not statistically significant (see Table 18 in section 2.6(d)). In mice, no treatment-related thyroid tumours were observed in either sex at any dose. An evaluation of the weight of evidence to support a non-mutagenic MOA for thyroid follicular cell tumours in rats is summarized below.

A1.2 Summary description of the postulated mode of action

Hepatic microsomal enzyme induction (UDPGT) by the chronic administration of xenobiotic chemicals leading to thyroid follicular cell neoplasms is a well-known pathway in rodents (McClain et al., 1988; Capen, 2007). Long-term exposure of rats to a wide variety of different chemicals may induce UDPGT pathways and result in chronic stimulation of thyroid by increased TSH levels. The chronic stimulation of the thyroid by increased TSH often results in a greater risk of developing thyroid follicular cell tumours in rats. In addition, T_4 metabolism takes place very rapidly in rats because of the absence of T_4 binding globulin in this species (McClain et al., 1988; Capen, 2007). This tumour development pathway is rodent specific and not relevant to humans. This process is a non-genotoxic mitogenic MOA. A possibility of direct effects of etofenprox on the thyroid similar to those of anti-thyroid substances was excluded.

A.1.3 Key events in the mode of action (Figure A1)

1. Induction of hepatic microsomal enzyme, UDPGT

In the 4-week study in rats, hepatic microsomal UDPGT activities using both the 4-MUGT and p-NPGT methods were significantly increased in both sexes at 20 000 ppm and in males at 5000 ppm at week 2. The effects showed reversibility, but remained 4 weeks after withdrawal. Hepatic microsomal UDPGT activity using *p*-nitrophenol as the acceptor substrate was significantly increased in both sexes at 5000 ppm and higher for 2 weeks. The effects remained at the end of the recovery period. The effect of treatment on TP activity was equivocal (Smith, 2003a).

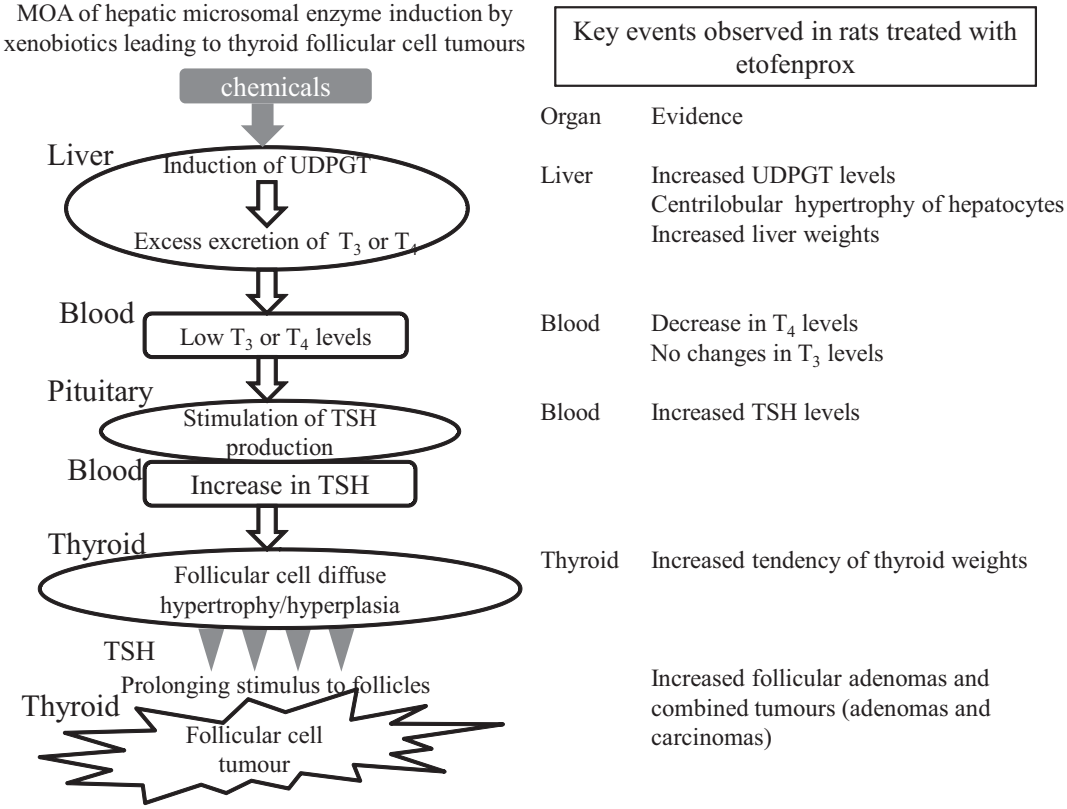
2. Decreases in T_3 or T_4 levels in the blood

In the 4-week dietary study, serum T_4 concentration was significantly lower in males at 20 000 ppm for 2 and 4 weeks. In females, an effect was not apparent (Smith, 2003a). There was no effect of 2 or 4 weeks of treatment on serum T_3 concentration at the same doses. In the dietary 90-day toxicity study in rats, T_4 concentration was significantly decreased in males at 10 800 ppm at weeks 6 and 13. In the carcinogenicity study in rats, T_3 and T_4 levels were comparable to those in the control group (Green et al., 1986a).

3. An increase in TSH in the blood

Serum TSH activity was significantly increased in both sexes at 20 000 ppm after 2 weeks of treatment and in females at 5000 and 20 000 ppm after 4 weeks in the 4-week dietary study (Smith,

Figure A1. Key events in the mode of action for follicular cell tumours in the thyroid in rats



2003a). Furthermore, the activity in both sexes at all dose levels was higher than the control value, but not significantly. Based on the consistency of these differences, the changes suggest a treatment-related increase.

4. Cell proliferation activity of follicular epithelium in the thyroid

There were no statistically significant increases in hepatic or thyroid cell proliferation rates, as measured by the BrdU labelling indices, in either sex at any dose and treatment regime with etofenprox. Hepatic labelling indices were comparable to control values in all groups and in both sexes. In the thyroid, BrdU-positive cells were slightly increased in males at weeks 2 and 4, but not statistically significantly. In females, the labelling index was significantly depressed at 20 000 ppm at week 2 only, which was considered an incidental finding, because the value was within the concurrent control range.

5. Increases in thyroid weights

The absolute thyroid weights were increased in both sexes of F_0, F_{1b} and F_{2b} adults except for F_{1b} females at 4900 ppm in the multigeneration study in rats (Cozens et al., 1985c). The increase was not apparent in other rat studies.

6. Liver hypertrophy

Corresponding to the increase in liver weights, centrilobular hypertrophy of hepatocytes was observed in the rat 90-day study (Green et al., 1983a), rat carcinogenicity study (Green et al., 1986a) and multigeneration studies at the highest doses (Cozens et al., 1985c).

A1.4 Dose–response relationships

Most of the key events in the MOA were observed in rats at the tumorigenic dose of 4900 ppm and higher. In the 4-week dietary study on thyroid function and hepatic microsomal enzyme induction in rats, induction of UDPGT in the liver, decreases in T_4 and increases in TSH were detected at a higher dose of 20 000 ppm (Smith, 2003a). Therefore, there is clear evidence of a dose–response relationship.

A1.5 Temporal association

The operating principle for the key events in the MOA analysis is that the key events should occur temporally before the appearance of tumours. The identified key events all occur prior to the observation of follicular cell tumours in the thyroid. Hepatic microsomal induction of UDPGT and cell proliferation in the follicular cells occurred within 2 weeks after repeated dosing. Some of the key events, such as increased liver weight, histopathological changes, hepatocellular hypertrophy, decreased T_4 levels and increased TSH levels in the blood, vacuolation and single-cell necrosis leading to focal hepatocytic hyperplasia, were observed in a 90-day toxicity study in rats (Green et al., 1983a) and also at interim sacrifice in a carcinogenicity study in rats (Green et al., 1986a), indicating that these key events occurred prior to tumours seen at terminal sacrifice in the carcinogenicity study in rats.

A1.6 Strengths, consistency, specificity of association of tumour response with key events

Hepatic microsomal enzyme induction by the chronic administration of xenobiotic chemicals leading to thyroid follicular cell hyperplasia and neoplasia is established as a well-known tumour development pathway in rodents (Capen, 2007). Many chemicals, including phenobarbital, calcium channel blockers or spironolactone, are reported to induce thyroid tumours through the same pathway. Most of the key events of these xenobiotics, described in Figure A1 above, are common to etofenprox. Thus, the MOA proposed for etofenprox is the same as that for other chemicals reported to induce thyroid follicular cell tumours through hepatic microsomal enzyme induction (Capen, 2007). There is good consistency in the results across multiple toxicity studies for etofenprox. The findings were induction of UDPGT activity in the liver and decreased T_4 (Green et al., 1983a, 1986a; Cozens et al., 1985c). In addition, this tumour response is also known to cause thyroid tumours in mice, although thyroid tumours were not induced in the carcinogenicity study in mice treated with etofenprox (Green et al., 1986b).

A1.7 Alternative modes of action

The genetic toxicity battery indicates that etofenprox is not acting via a genotoxic mode of carcinogenic action. There are two pathways in follicular cell tumour development in the thyroid in rodents: direct and indirect action of test materials. The overall weight of evidence from short-term and carcinogenicity studies, a multigeneration study and a 4-week study on thyroid function and hepatic microsomal enzyme induction of etofenprox demonstrated that the direct target of etofenprox is induction of UDPGT activity in the liver, but not the thyroid.

A1.8 Conclusion on the postulated mode of action in animals

The overall weight of evidence supports a non-genotoxic mitogenic MOA for etofenprox through indirect effects on the thyroid. Etofenprox increased UDPGT in the liver, leading to excess excretion of T_4 from the blood. Decreased serum T_4 levels continuously stimulate TSH production, resulting in follicular cell tumour development. Rodents are sensitive to the induction of thyroid follicular cell tumours, first because of easy induction of UDPGT in rodent species and second because of rapid T_4 metabolism in the absence of T_4 binding globulin in the serum of rodents. Direct effects of etofenprox on thyroid are excluded.

A1.9 Relevance of etofenprox-induced tumours to humans

Rodents are sensitive to the induction of thyroid follicular cell tumours, because of the easy induction of UDPGT in rodent species, resulting in direct thyroid tumour induction, which is described above. In addition, rapid T_4 metabolism is not plausible for humans, because T_4 binding globulin is absent in the serum of rodents, but not in that of humans (Capen, 2007). Therefore, hepatic microsomal enzyme induction by etofenprox leading to thyroid follicular cell tumours is rodent specific and not relevant to humans.

FLUTRIAFOL

First draft prepared by
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Explanation

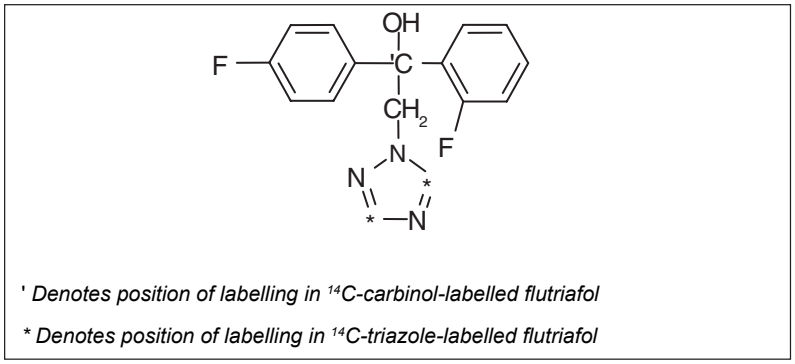
Flutriafol is the International Organization for Standardization (ISO)–approved name for (*RS*)-2,4′-difluoro-α-(1H-1,2,4-triazol-1-ylmethyl)benzhydryl alcohol (International Union of Pure and Applied Chemistry), which has the Chemical Abstracts Service No. 76674-21-0. Flutriafol is a contact and systemic fungicide belonging to the triazole class. It is used on a wide range of cereal crops and as a seed treatment. Its fungicidal mechanism of action is the inhibition of ergosterol biosynthesis and thus disruption of fungal cell wall synthesis.

Flutriafol was reviewed for the first time by the Joint FAO/WHO Meeting on Pesticide Residues at the request of the Codex Committee on Pesticide Residues. All critical studies contained statements of compliance with good laboratory practice.

Evaluation for acceptable daily intake

The structure of flutriafol showing the positions of the radioactive labels is given in Figure 1.

Figure 1. Structure of flutriafol showing position of carbinol and triazole labels



1. Biochemical aspects

1.1 Absorption, distribution and excretion

(a) Oral route

Rats

Alderley Park (Wistar-derived) albino rats (five of each sex per group) were gavaged with a single dose of ^{14}C-carbinol-labelled flutriafol (specific activity 2.17 GBq/mmol / 373.03 kBq/mg; radiochemical purity > 98%) suspended in polyethylene glycol 600 (PEG600) at a dose level of 5 mg/kg body weight (bw). Excreta were collected up to termination at 7 days following administration. The levels of radioactivity in excreta and tissue samples were measured by liquid scintillation counting (LSC) following appropriate processing. In a whole-body autoradiography phase, two additional animals (one of each sex) were gavaged with a single dose of the test material (1.898 MBq/mg) and terminated at 48 hours after dosing. Longitudinal sagittal sections were cut and prepared for autoradiography. Radioactivity was also determined in urine, faeces and exhaled carbon dioxide.

In the main study phase, radioactivity was excreted rapidly (~95% within 48 hours) and approximately equally in urine and faeces, although faecal excretion was slightly greater in males (~52%) than in females (~45%). Little radioactivity was excreted after 72 hours. Negligible amounts of radioactivity were excreted as carbon dioxide, and total residual radioactivity in the carcass at 168 hours following dosing was low (< 1%). At 168 hours post-dosing, highest concentrations were in whole blood (0.1–0.2 µg equivalents [eq] per gram), with no other tissues having levels above 0.1 µg eq/g.

Whole-body autoradiography at 48 hours after dosing indicated that the bulk of the administered radioactivity was present in the contents of the gastrointestinal tract between the stomach and rectum, with lesser amounts present in the liver and kidney. Liver radioactivity appeared to be greater in males than in females, and the liver in males displayed a reticular appearance (interpreted as being indicative of preferential absorption at a particular area of the lobule), whereas hepatic distribution in females was more homogeneous. Renal radioactivity was observed at the cortico-medullary junction

Table 1. Metabolism study design

Radiolabel	Dose level (mg/kg bw)	No. of animals		Bile duct cannulation	Samples
		Males	Females		
^{14}C-carbinol	250	—	6	—	Urine, faeces
^{14}C-carbinol	250	2	2	✓	Bile, urine
^{14}C-carbinol	5	2	2	✓	Bile, urine, faeces
^{14}C-triazole	5	2	2	✓	Bile, urine, faeces

From Jones & Ladd (1986)

in both sexes. Traces of radioactivity were present in the adrenals in the female. No significant levels of radioactivity were noted in other organs (Jones et al., 1982).

Alpk:AP rats were gavaged with single doses of ^{14}C-carbinol- or ^{14}C-triazole-radiolabelled test material (^{14}C-carbinol label specific activity 2.2 GBq/mmol; ^{14}C-triazole label specific activity 2.2 GBq/mmol) at a dose level of 5 or 250 mg/kg bw in PEG600 (Table 1). Excreta were collected for 72 hours following dosing, and radioactivity was measured by LSC.

In the low-dose animals, excretion was rapid and primarily via the bile. Biliary excretion was extensive (~60–80% of the administered dose), with urinary excretion of approximately 10–25%; faecal excretion was low (< 10%) (Table 2). There were some differences in results between the sexes and radiolabel positions, but no obvious patterns. The excretion profile in bile indicates that the large majority of faecal radioactivity excreted by rats in the previous study (Jones et al., 1982) is likely to be biliary in origin.

At the high dose level, total recovery of radioactivity in all groups was incomplete (80–90%) at the 72-hour time point, indicating some saturation of absorption and/or excretion. Excretion of radioactivity in non-cannulated female rats administered the carbinol label was primarily via urine (Table 2), showing reasonable consistency with the non-cannulated females in the previous study (Jones et al., 1982). In bile duct–cannulated animals administered the triazole label, biliary excretion was more extensive in males (~71%) than in females (~47%). Urinary excretion was more extensive in females (~31%) than in males (~19%) (Table 2). The findings indicate that biliary excretion might be saturated in females administered a single high dose of 250 mg/kg bw.

An oral absorption value in the rat of greater than 90% can be estimated for flutriafol based on figures for the biliary and urinary excretion of radioactivity at the low dose level in this study (Jones & Ladd, 1986).

Groups of Sprague-Dawley rats (four of each sex) were gavaged with either a single dose of 250 mg/kg bw or 14 consecutive daily doses of ^{14}C-carbinol-labelled flutriafol (specific activity 1.835 GBq/mmol; suspended in PEG600) at 5 mg/kg bw. Urine and faeces were collected for up to 168 hours following the final dose; radioactivity was analysed by LSC. Following a single oral dose of flutriafol at 250 mg/kg bw, excretion was primarily urinary (~61% and ~68% in males and females, respectively), although faecal excretion was also a significant route (~33% and ~27% in males and females, respectively). The majority of the administered radioactivity was excreted within 72 hours of dosing.

The excretion of radioactivity within 24 hours of the administration of the first of the multiple low doses was proportionally greater than that seen within 24 hours of administration of the single high dose (Table 3), although the absolute proportions of the administered radiolabel excreted via each route were independent of dose level.

Residual levels in the carcass and tissue samples were low (< 1% of the administered dose), with highest concentrations in whole blood, 40- to 100-fold above levels in plasma, indicating concentration in and/or binding to blood cells (Millais, 2004).

Table 2. Excretion of radioactivity in rats administered flutriafol

Sampling time (h)	Radioactivity (% of administered dose)							
	5 mg/kg bw				250 mg/kg bw			
	^{14}C-carbinol		^{14}C-triazole		^{14}C-carbinol		^{14}C-triazole	
	Males	Females	Males	Females	Males	Females	Males	Females
Urine								
0–24	7.14	11.06	9.06	13.98	—	16.69	4.21	6.12
24–48	4.31	13.66	11.17	9.68	—	26.97	12.99	11.13
48–72	0.46	0.27	1.75	0.43	—	17.21	1.58	14.14
Total	11.91	24.97	21.97	24.08	—	60.85	18.78	31.38
Faeces								
0–24	0.08	0.25	1.27	0.22	—	1.45	—	—
24–48	0.44	2.30	4.66	0.36	—	8.46	—	—
48–72	0.32	1.34	4.47	1.22	—	11.89	—	—
Total	0.84	3.89	10.40	1.79	—	21.79	—	—
Bile								
0–24	63.91	45.44	42.64	56.79	—	—	23.80	6.08
24–48	15.22	12.75	19.62	15.96	—	—	44.15	14.55
48–72	0.13	0.15	0.46	0.28	—	—	3.00	26.25
Total	79.26	58.34	62.72	72.01	—	—	70.95	46.87
Total								
0–24	71.13	56.75	52.96	70.98	—	18.12	28.01	12.20
24–48	19.97	28.69	35.45	25.99	—	35.41	57.14	25.68
48–72	0.91	1.76	6.68	1.92	—	29.11	4.58	40.38
Total	92.00	87.21	95.08	98.89	—	82.64	89.72	78.25

From Jones & Ladd (1986)

(b) Dermal route

In vitro

In an in vitro dermal absorption study of [^{14}C]flutriafol in a 125 g/l suspension concentrate, there was evidence of the extent of dermal absorption being limited by the applied concentration. Absorption of flutriafol from the undiluted product was low through both rat and human skin samples, but increased markedly on dilution. Dermal absorption values were 0.3%, 12% and 46% for human skin and 2.3%, 36% and 53% for rat skin for the concentrate, 0.79 g/l and 0.025 g/l dilutions, respectively, based on receptor fluid and application site after tape stripping (deLigt, 2004).

Rats

In an in vivo dermal absorption study of [^{14}C]flutriafol in a 125 g/l suspension concentrate, F344 rats were exposed to the undiluted product and 0.79 and 0.025 g/l dilutions for 8 hours. Urine and faeces were collected until sacrifice at 24, 96 or 216 hours following application (four rats per group per time point). Skin at the application site was shaved and tape-stripped (10 strips) at termination. Recovery in all groups was acceptable (means 93–99%). Absorption of radiolabel from both dilutions was essentially complete within 72 hours; with the concentrate, the rate of absorption was consistent over time. The amount of radioactivity excreted was relatively low in all groups, but increased with dilution. Faecal excretion of radioactivity was greater, up to 2-fold, than urinary excretion. The levels

Table 3. Excretion of radioactivity in rats receiving a single high or repeated low doses of flutriafol

Sampling time (h)	Radioactivity (% of administered dose)									
	Day 1		Day 1		Day 5		Day 10		Day 14	
	High dose		Repeated low dose							
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
Urine										
0–6	1.89	4.37	20.4	21.4	15.9	27.5	22.4	29.6	11.9	27.6
6–24	17.8	17.7	29.8	32.6	34.0	27.5	28.4	27.5	36.7	33.1
24–48	29.8	34.1	—	—	—	—	—	—	9.67	4.59
48–72	7.64	9.38	—	—	—	—	—	—	3.37	1.26
72–96	2.22	1.19	—	—	—	—	—	—	1.26	0.71
96–120	0.74	0.42	—	—	—	—	—	—	0.56	0.40
120–144	0.38	0.25	—	—	—	—	—	—	0.43	0.35
144–168	0.22	0.12	—	—	—	—	—	—	0.28	0.24
Total	60.6	67.5	50.2	53.9	49.8	54.9	50.8	57.1	64.2	68.2
Faeces										
0–6	0.08	0.19	0.09	0.01	0.66	1.72	1.17	2.63	0.65	1.08
6–24	3.91	4.17	29.4	33.1	35.8	35.0	30.2	37.1	30.8	31.6
24–48	14.1	12.6	—	—	—	—	—	—	14.1	6.1
48–72	11.6	8.5	—	—	—	—	—	—	4.84	0.97
72–96	2.20	1.14	—	—	—	—	—	—	1.72	0.44
96–120	0.70	0.22	—	—	—	—	—	—	1.11	0.27
120–144	0.26	0.10	—	—	—	—	—	—	0.88	0.21
144–168	0.17	0.05	—	—	—	—	—	—	0.52	0.23
Total	33.1	26.9	29.5	33.1	36.4	36.7	31.4	39.8	54.7	40.8
Cage wash										
Total	2.79	4.29	2.01	2.25	1.74	2.07	2.73	2.25	3.41	2.92
Carcass	0.25	0.23	—	—	—	—	—	—	2.73	2.25
Recovery	96.8	99.0	81.6[a]	89.3[a]	88.0[a]	93.7[a]	84.9[a]	99.2[a]	125[b]	115[b]

From Millais (2004)

[a] Recovery of radioactivity during the 24 hours following dosing, relative to the administered dose.

[b] Recovery of radioactivity during the 168 hours following dosing, relative to a single administered dose.

of radioactivity in whole blood and plasma are consistent with binding to red blood cells, which is consistent with the results of oral dosing studies. Dermal absorption values of 1%, 10% and 12% were derived for the undiluted formulation, 0.79 g/l and 0.025 g/l dilutions, respectively, based on excretion, residues in carcass and residues in application site after tape stripping (deBie & Grossouw, 2004).

1.2 Biotransformation

Rats

Samples of urine, bile and faeces from the study by Jones & Ladd (1986) were analysed for metabolites of flutriafol by comparative thin-layer chromatography (TLC), high-performance liquid

chromatography (HPLC), gas chromatography–mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR).

Analysis of methanol extracts of urine (containing > 96% of the urinary radioactivity) indicated extensive metabolism, with only trace amounts of unchanged parent compound detected. The metabolite profile and the relative proportions of metabolites were reported to be similar in urine collected from rats at the low and high dose levels; however, further details were not reported. The urinary metabolite profiles in bile duct–cannulated rats were stated to be similar to those in non-cannulated rats. The primary reactions were hydroxylation on the 2-fluorophenyl ring followed by conjugation. There was evidence of only limited cleavage to release the triazole moiety.

Chromatographic analysis of bile samples from rats administered either the low or high dose level showed that more than 95% of biliary radioactivity corresponded to polar metabolites believed to be conjugates. TLC of bile samples from rats administered either the ^{14}C-carbinol- or the ^{14}C-triazole-labelled test material did not show any evidence of cleavage of the triazole group. Acid hydrolysis resulted in a metabolic profile stated to be similar to that seen in urine. The profile of faecal metabolites was also reported to be similar to that in urine (Jones & Ladd, 1986).

Samples from the study of Millais (2004) were analysed using HPLC, NMR and liquid chromatography with mass spectrometry (LC-MS) and tandem mass spectrometry (LC-MS/MS). Urinary metabolites were further characterized following treatment with β-glucuronidase/sulfatase. HPLC revealed a total of 19 metabolite regions (M1–19) in faecal extracts and urine samples; the major components were characterized.

The predominant urinary metabolite regions were M3, M5, M6, M8 and M15 (Tables 4 and 5), with no unchanged parent detected in the urine (Table 5). Metabolite profiles in the two sexes were broadly similar, although at 250 mg/kg bw, higher levels of unconjugated M15 were detected in the urine of males (15.5%) than in the urine of females (6.6%). Enzyme treatment increased the proportions of M4, M12, M14, M15 (markedly) and M18, indicating that these regions consisted largely of unconjugated metabolites (Table 5). Differences in metabolite patterns in high- and low-dose groups were mainly due to the level of conjugation. The metabolism was postulated to be initial oxidation and conjugation on the 2-fluorophenyl ring. The metabolite profile in faeces was broadly consistent with that in urine.

The proposed metabolic pathways for flutriafol in rats are shown in Figure 2.

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

Flutriafol is of moderate acute toxicity via the oral route, but of low toxicity via the dermal and inhalation routes (Table 6).

(b) Dermal and ocular irritation and dermal sensitization

Flutriafol (in 0.9% saline) was not irritating to rat or rabbit skin (Henderson & Parkinson, 1982; Sanders, 2005a). Flutriafol was a mild irritant to rabbit eyes; all effects had resolved within 72 hours (Henderson & Parkinson, 1982; Durando, 2007). Flutriafol did not produce any evidence of skin sensitization in a Magnusson and Kligman assay in guinea-pigs (Barber & Parkinson, 1982) or in a local lymph node assay in mice (Sanders, 2005b).

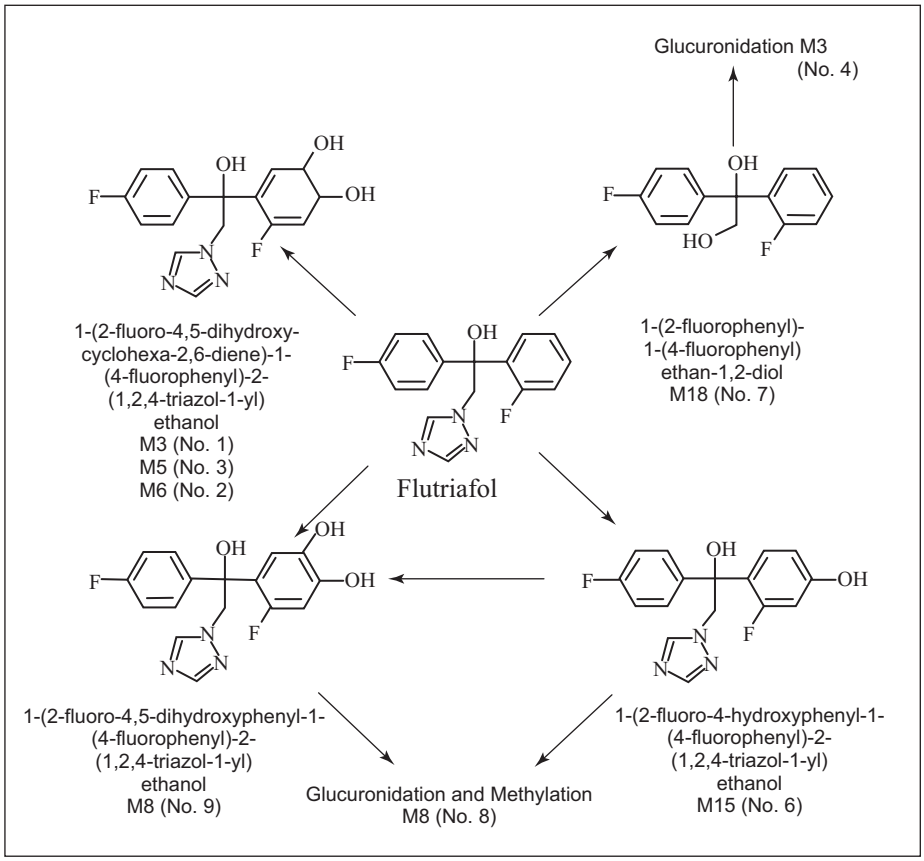
Table 4. Identity of major flutriafol metabolites

HPLC region	Chemical name
M3	1-(2-Fluoro-4,5-*cis*-dihydroxy-cyclohexa-2,6-diene)-1-(4-fluorophenyl)-2-(1,2,4-triazol-1-yl) ethanol
	1-(2-Fluorophenyl)-1-(4-fluorophenyl) ethan-1,2-diol glucuronide
M5	(*S*,*S*)-1-(2-Fluoro-4,5-*trans*-dihydroxy-cyclohexa-2,6-diene)-1-(4-fluorophenyl)-2-(1,2,4-triazol-1-yl) ethanol
M6	(*R*,*R*)-1-(2-Fluoro-4,5-*trans*-dihydroxy-cyclohexa-2,6-diene)-1-(4-fluorophenyl)-2-(1,2,4-triazol-1-yl) ethanol
M8	1-(2-Fluoro-4-hydroxyphenyl)-1-(4-fluorophenyl)-2-(1,2,4-triazol-1-yl) ethanol glucuronide
	1-(2-Fluoro-4,5-dihydroxyphenyl)-1-(4-fluorophenyl)-2-(1,2,4-triazol-1-yl) ethanol glucuronide
M15	1-(2-Fluoro-4-hydroxy-5-methoxyphenyl)-1-(4-fluorophenyl)-2-(1,2,4-triazol-1-yl) ethanol
	1-(2-Fluoro-4-hydroxyphenyl)-1-(4-fluorophenyl)-2-(1,2,4-triazol-1-yl) ethanol
M18	1-(2-Fluorophenyl)-1-(4-fluorophenyl) ethan-1,2-diol

Table 5. Urinary metabolites in rats administered flutriafol

Metabolite region	Enzyme treated	Radioactivity (% of daily dose)									
		Repeated low dose								High dose	
		Males				Females				Males	Females
		Day 1	Day 5	Day 10	Day 14	Day 1	Day 5	Day 10	Day 14		
M3	−	4.8	4.9	5.2	5.0	5.1	6.0	6.3	7.8	6.6	8.7
	+	3.5	3.7	3.2	2.7	6.4	6.2	6.9	6.2	< 0.1	< 0.1
M4	−	0.3	0.2	0.5	0.6	0.5	0.3	0.3	0.3	< 0.1	< 0.1
	+	1.5	2.7	2.6	2.3	0.9	0.7	0.8	1.2	< 0.1	< 0.1
M5	−	6.9	5.9	5.8	6.8	12.0	12.2	13.4	11.9	6.4	7.5
	+	6.7	5.5	5.6	6.2	12.3	13.0	13.8	11.9	10.4	5.0
M6	−	5.6	6.2	6.5	6.5	8.6	9.7	9.1	9.9	6.6	9.0
	+	5.4	3.8	5.1	5.6	7.8	7.6	7.7	10.1	< 0.1	< 0.1
M8	−	10.4	9.7	8.6	7.6	9.5	10.5	10.5	12.7	4.6	8.6
	+	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1	8.5	13.7
M14	−	0.1	0.1	0.4	0.5	0.1	0.2	0.1	0.1	1.7	3.2
	+	2.9	2.6	2.4	2.7	4.0	4.6	3.9	2.8	< 0.1	4.5
M15	−	3.5	5.3	4.3	4.5	1.2	1.4	1.3	1.3	15.2	6.6
	+	25.2	25.0	24.9	22.1	15.6	17.7	18.9	21.4	16.5	15.5
Flutriafol	−	0.2	< 0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	+	0.1	0.1	< 0.1	< 0.1	0.1	< 0.1	< 0.1	0.2	< 0.1	< 0.1
M18	−	0.1	0.2	0.4	0.4	0.1	< 0.1	0.1	< 0.1	2.5	1.6
	+	1.7	2.5	2.4	2.0	0.4	0.9	0.7	1.0	9.1	4.6

Figure 2. Proposed metabolism of flutriafol in rats



2.2 Short-term studies of toxicity

(a) Oral administration

Mice

Alderley Park (C57BL/10J/Alpk) mice (five of each sex per group) received flutriafol (unspecified) in the diet at a concentration of 0, 50, 150, 500 or 1500 parts per million (ppm) for 29 days. Routine observations and investigations were performed. Gross necropsy was performed on all animals when weights of the brain, heart, liver, kidneys, spleen and testes were recorded. A comprehensive range of tissues from control, 500 and 1500 ppm animals were examined histopathologically; investigations in the other dose groups were limited to the liver, testes, epididymides, ovaries, cervix, uterus and adrenals. The level of reporting in this range-finding study is limited.

Dietary concentrations were not confirmed analytically, and doses of flutriafol were estimated to be 0, 7.5, 22.5, 75 and 225 mg/kg bw per day. Most top-dose animals were found dead during the study. Signs of toxicity at the top dose level included subdued behaviour, hunched posture, emaciation, shaking, piloerection and urinary staining. No deaths occurred and no signs of toxicity were observed at lower dose levels. Reduced body weight gain was evident at and above 500 ppm and to a lesser extent in males at 150 ppm (Table 7). Dose-related increases in absolute and relative liver weights were evident at 150 and 500 ppm (Table 7). Gross necropsy did not reveal any treatment-related findings in either sex at the top dose level; liver enlargement was observed in a number of animals at 500 ppm (further details not reported). Histopathology revealed hepatotoxicity in both sexes at all dose levels (Table 7).

A no-observed-adverse-effect level (NOAEL) cannot be determined for this range-finding study as a result of histopathology (lipid accumulation in the liver) of both sexes at 50 ppm (equivalent to 7.5 mg/kg bw per day), the lowest dose tested (Hodge & Robinson, 1985).

Table 6. Acute toxicity studies with flutriafol

Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	LC_{50} (mg/l)	Purity (%); batch No.	Vehicle	Reference
Rat	AlPk	M F	Oral	1140 1480	—	93; P10	"Lissatan AC"	Henderson & Parkinson (1982)
Mouse	AlPk	M F	Oral	365 179	—	93; P10	"Lissatan AC"	Henderson & Parkinson (1982)
Rabbit	New Zealand White	F	Oral	300–400	—	93; P10	"Lissatan AC"	Henderson & Parkinson (1982)
Guinea-pig	Dunkin Hartley	M	Oral	300–400	—	93; P10	"Lissatan AC"	Henderson & Parkinson (1982)
Rat	AlPk	M F	Dermal	> 1000 (HDT)	—	93; P10	PEG300	Henderson & Parkinson (1982)
Rabbit	New Zealand White	M F	Dermal	> 2000 (HDT)	—	93; P10	PEG300	Henderson & Parkinson (1982)
Rat	AlPk	M F	Inhalation (nose only)	—	> 3.5 (MMAD 16 μm)	93; P10	None (aerosol)	Henderson & Parkinson (1982)
Rat	Sprague-Dawley	M F	Inhalation (nose only)	—	> 5.2 (MMAD 4 μm)	95.1; UPL BX1	None (aerosol)	Griffiths (2005)

F, female; HDT, highest dose tested; LC_{50}, median lethal concentration; LD_{50}, median lethal dose; M, male; MMAD, mass median aerodynamic diameter; PEG, polyethylene glycol

Rats

Alderley Park (Wistar derived) rats (eight of each sex per group) were administered flutriafol (purity 97%; batch No. P7) in the diet at a concentration of 0, 100, 300, 800, 2000 or 5000 ppm for 28 days. Standard observations and investigations were performed. A comprehensive range of tissues from control and top dose level animals were examined histopathologically, including oil red O staining of liver sections; investigations in intermediate-dose groups were limited to grossly abnormal tissues. Further liver samples from various groups were investigated by electron microscopy; peroxisome and smooth endoplasmic reticulum volumes were determined. Hepatic aminopyrine *N*-demethylase activity was also measured at termination (six rats of each sex per group).

Achieved doses were 0, 11, 34, 86, 215 and 517 mg/kg bw per day. One top-dose female was found dead on day 7. All top dose level animals were noted to be smaller and thinner than controls from day 4; other signs of toxicity in this group included ptosis, hunched posture, chromodacryorrhoea and piloerection. Signs of toxicity were not apparent at lower dose levels. Weight gain by both sexes at the top dose level was markedly reduced; feed consumption by these animals was lower during weeks 1–2 but was comparable to that of controls during weeks 3–4. Feed utilization efficiency was significantly lower (~50%) in both sexes at the top dose level and was also slightly (~10%) lower in 2000 ppm males. A range of haematology and clinical chemistry changes were noted at 2000 and 5000 ppm. Aminopyrine *N*-demethylase activity was increased in all groups in a dose-related manner (males, 19, 21, 27, 30, 36 and 26 μmol/h per gram; females 7, 8, 11, 18, 27 and 26 μmol/h per gram).

The weights of a number of organs at the top dose level were affected by the markedly lower body weights in this group. Mean absolute liver weights were significantly higher in males at and above

Table 7. Twenty-nine-day mouse study with flutriafol: summary of findings

Parameter	Sex	Dietary concentration (ppm)				
		0	50	150	500	1500
Weight gain (g)						
- weeks 0–1	Male	4.4	3.4	2.6	0.6*	—
	Female	3.0	2.6	2.0	1.0*	−1.3**
- weeks 0–4	Male	8.8	8.9	7.1	3.6**	—
	Female	5.0	5.3	5.2	2.3*	—
Histopathology in liver (n = 5)[a]						
- hepatocyte hypertrophy	Male	0	3 [3, 0, 0]	5 [0, 5, 0]	5 [2, 3, 0]	—
	Female	0	0	0	0	—
- cytoplasmic vacuolation	Male	0	0	3 [3, 0, 0]	5 [0, 3, 2]	—
	Female	0	0	0	2 [2, 0, 0]	—
- lipid accumulation	Male	0	2 [2, 0, 0]	4 [3, 1, 0]	5 [0, 5, 0]	—
	Female	0	3 [3, 0, 0]	4 [4, 0, 0]	5 [2, 3, 0]	2 [2, 0, 0]

From Hodge & Robinson (1985)
* $P < 0.05$; ** $P < 0.01$ (Student's t-test)
[a] Incidence [severity]. Laboratory severity scale: minimal, slight, moderate.

800 ppm and in females at and above 300 ppm (Table 8). Gross pathology revealed pale, enlarged, granular and/or yellow-brown livers with an accentuated lobular pattern at the top dose level; lower incidences of these findings were also noted at 2000 ppm. Microscopically, evidence of liver toxicity was seen at and above 2000 ppm and was characterized by centrilobular hepatocyte hypertrophy and vacuolation, fatty change and hydropic degeneration (Table 8). Hepatocyte hypertrophy was seen in males at 800 ppm. Electron microscopy revealed significantly increased hepatic smooth endoplasmic reticulum volume in 2000 ppm males, consistent with microsomal enzyme induction; hepatic peroxisome volume in 100 ppm females was comparable to that of controls. Reduced spermatogenesis, atrophy of the prostate and contracted tubules in the seminal vesicles were observed in top-dose males. Slight focal haemorrhage of the thymus was noted in some top-dose females. Oil red O staining revealed moderate to marked deposition of triglycerides in the liver of top dose level animals.

A NOAEL of 300 ppm (equal to 34 mg/kg bw per day) can be determined for this study, based on the marked increase (> 15%) in absolute and relative liver weights at 800 ppm (equal to 86 mg/kg bw per day). The statistically significantly higher relative liver weights in males at 100 ppm and in both sexes at 300 ppm are less than 10% in relative terms and are not considered to be adverse. The significantly elevated hepatic aminopyrine *N*-demethylase activity in both sexes at 300 ppm is considered to be adaptive rather than adverse (Milburn, 1982).

Alderley Park (Wistar-derived) rats (20 of each sex per group) received flutriafol (purity 93%; batch No. P10) in the diet at a concentration of 0, 20, 200 or 2000 ppm for 90 days. Routine observations and investigations were performed. All animals were subjected to gross necropsy, and main organs were weighed. A comprehensive range of tissues from control and top dose level animals were examined histopathologically; investigations in the intermediate-dose groups were limited to the liver, kidney, heart, lungs, gonads, uterus, epididymides, prostate, seminal vesicles and grossly abnormal tissues. Additional liver samples (from six rats of each sex per group) were investigated using electron microscopy; hepatic aminopyrine *N*-demethylase activity was measured in a further 10 samples from each sex per group.

Achieved doses were 0, 1.4, 13 and 148 mg/kg bw per day. Two low-dose males died during the study; neither death was considered to be treatment related. No signs of toxicity were observed during

Table 8. Twenty-eight-day rat study: liver weights and liver histopathology

Parameter	Sex	Dietary concentration (ppm)					
		0	100	300	800	2000	5000
Liver weights							
- absolute (g)	Male	14.24	15.80	15.96	17.09*	19.52**	16.81*
	Female	8.63	9.14	9.68**	10.10**	13.27**	15.43**
Relative (%)	Male	4.66	5.09**	5.07*	5.61**	7.21**	10.13**
	Female	4.28	4.49	4.66**	5.06**	6.60**	10.17**
Liver histopathology (n = 8)							
- hepatocyte hypertrophy	Male	0	0	1	6	8	8
	Female	0	0	0	0	5	7
- fatty change	Male	0	0	0	0	8	8
	Female	0	0	0	0	8	7
- hydropic degeneration	Male	0	0	0	0	3	2
	Female	0	0	0	0	0	5
- smooth endoplasmic reticulum volume (%)	Male	25.4	27.6	—	—	57.3*	—
	Female	20.9	22.5	—	—	—	—

From Milburn (1982)
* $P < 0.05$; ** $P < 0.01$ (Student's t-test)

the study period. Ophthalmoscopy and urinalysis did not reveal any treatment-related findings. Mean terminal body weights of both sexes at the top dose level were lower (~10%) than those of controls as a result of significantly lower weight gain from week 1 (Table 9). Overall feed consumption was slightly (but significantly) lower in all treated groups in a dose-related fashion.

Haematology revealed significant effects on erythrocyte parameters consistent with mild anaemia in both sexes at the top dose level at weeks 4 and 13. A range of clinical chemistry parameters consistent with hepatotoxicity were statistically significantly altered in top-dose animals, together with increases in blood urea nitrogen in top-dose females. Hepatic aminopyrine *N*-demethylase activity was significantly elevated in both sexes at and above 200 ppm.

The mean absolute and body weight–adjusted weights of a number of organs at the top dose level were influenced by the lower terminal body weights of both sexes. Marked increases in relative and absolute liver weights were seen at 2000 ppm (Table 9). The liver weight effects in 200 ppm females represent an increase of less than 10% and are therefore not considered to be of toxicological significance in the absence of any evidence of hepatotoxicity in this group. Gross necropsy revealed increased incidences of enlarged/swollen and pale livers with well-defined surface reticulation in top-dose males. Microscopically, liver effects were characterized by centrilobular hepatocyte hypertrophy and marked diffuse fatty vacuolation in both sexes at the top dose level, with a single incidence of focal necrosis in one top-dose male. Electron microscopy revealed significantly greater smooth endoplasmic reticulum volume in the hepatocytes of both sexes and an increase in lipid levels in centrilobular hepatocytes, usually in the form of pale droplets at the top dose level. Also in the 200 ppm males, this took the form of numerous small dense droplets additionally to a moderate proliferation of smooth endoplasmic reticulum (Table 9).

The NOAEL is 200 ppm (equal to 13 mg/kg bw per day), based on the reduced weight gain, haematological and clinical chemistry effects and liver histopathology at the top dose level. The significantly increased aminopyrine *N*-demethylase activity, slightly increased liver weights and increased smooth endoplasmic reticulum volume seen at 200 ppm are considered not to be adverse effects (Pigott et al., 1982).

Table 9. Ninety-day rat study with flutriafol: body weights, feed consumption and liver pathology and histopathology

Parameter	Males				Females			
	Dietary concentration (ppm)							
	0	20	200	2000	0	20	200	2000
Weight (g)								
- week 13	494.5	492.7	489.3	439.9	271.5	278.3	276.3	243.0
Weight gain (g)								
- week 1	54.4	54.7	54.9	20.6**	34.1	33.4	32.6	18.5**
- week 4	194.2	196.0	196.7	151.4**	101.8	98.8	93.9**	81.9**
- week 13	360.8	367.2	360.9	308.2**	156.8	161.6	157.9	127.8**
Feed consumption (g)								
- weeks 1–13	2327	2237*	2182**	2159**	1693	1583**	1567**	1373**
Liver weight (g)								
- absolute	17.3	18.0	17.7	23.6**	9.2	9.7	9.9*	11.0**
- adjusted for body weight	16.6	17.2	17.2	25.4**	9.1	9.4	9.6*	11.7**
Gross pathology of liver (n = 20)								
- liver pale/white	0	0	0	12	0	0	0	0
- liver surface reticulation	0	0	0	6	0	0	0	0
- liver swollen/enlarged	0	0	0	7	0	0	0	0
Histopathology of liver								
- hepatocyte hypertrophy	0	0	0	17	0	0	0	8
- fatty change	3	4	6	19	2	1	2	6
- focal necrosis	0	0	0	1	0	0	0	0
- smooth endoplasmic reticulum (%)	28.9	27.8	41.8**	50.6**	24.4	26.4	27.6	51.4**

From Pigott et al. (1982)
* $P < 0.05$; ** $P < 0.01$ (Student's t-test)

Dogs

Beagle dogs (four of each sex per group) were administered flutriafol (purity 93%; batch No. P10) in capsules at a dose level of 0, 1, 5 or 15 mg/kg bw per day for 90 days. Routine observations and investigations were performed pre-test and during dosing. All animals were subjected to gross necropsy; weights of the main organs were recorded. A comprehensive range of tissues from control and top dose level animals were examined histopathologically; a smaller selection of organs from intermediate-dose groups was investigated. In addition, samples of heart, liver and adrenals from control and top dose level animals were stained with oil red O; samples of liver and spleen from all animals were stained with Prussian blue. Liver samples were analysed for aminopyrine *N*-demethylase activity.

No deaths occurred and no signs of toxicity were observed during the study period. Feed consumption was unaffected by treatment. The mean terminal body weight of top-dose females was significantly lower than that of controls as a result of an initial slight weight loss and subsequently significantly reduced weight gain. Initial weight gain by top-dose males was also significantly reduced (Table 10). A tendency to higher white blood cell counts was seen in both sexes at the top dose level. Erythrocyte parameters were unaffected by treatment. Clinical chemistry investigations revealed significantly elevated serum alkaline phosphatase (ALP) activity in both sexes at the top dose level at all time points. Triglyceride concentrations were higher in top-dose females at all time points,

significantly at week 13. Hepatic aminopyrine *N*-demethylase activity was significantly elevated in both sexes at and above 5 mg/kg bw per day. Urinalysis did not reveal any clearly treatment-related effects (Table 10).

Mean absolute (males) and relative liver weights (both sexes) were significantly higher at the top dose level (Table 10). Relative liver weight was also significantly higher in females at 1 mg/kg bw per day; however, the Meeting concluded that this value does not form part of a dose–response relationship and is therefore not considered to be related to treatment. Mean absolute and relative ovary weights were significantly lower in all treated groups of females; however, a dose–response relationship is not apparent at 1 and 5 mg/kg bw per day. The effects on ovary weight at the top dose level are considered to be treatment related and might be secondary to reduced weight gain in this group. Additionally, one outlier with a high mean ovary weight (1.258 g) is noted in controls; the group mean value for absolute ovary weight excluding this individual is 0.927 g. Gross necropsy did not reveal any treatment-related findings. Microscopically, a slight increase in the number of haemosiderin-laden Kupffer cells was noted in the livers of both sexes at the top dose level; increased splenic haemosiderin content was noted in top-dose males.

A NOAEL of 5 mg/kg bw per day can be determined for this study, based on a range of findings at the top dose level of 15 mg/kg bw per day. The significantly elevated aminopyrine *N*-demethylase activity in both sexes at and above 5 mg/kg bw per day is considered to represent an adaptive rather than an adverse effect. The significantly lower ovary weights in females at 1 and 5 mg/kg bw per day are not considered to be of clear toxicological significance in the absence of a dose–response relationship or histopathological correlates (Kalinowski et al., 1982).

Beagle dogs (four of each sex per group) were administered flutriafol (purity 93%; batch No. P10) in capsules at a dose level of 0, 1, 5 or 20 mg/kg bw per day for 1 year. Dogs were observed at least daily for clinical signs; more detailed physical examinations were performed weekly, and full veterinary examinations (including ophthalmoscopy) were performed pre-test and during weeks 13, 26, 39 and 52. Body weights and feed consumption were recorded weekly throughout the study period. Blood samples were taken pre-test and during weeks 4, 13, 26 and 52 for the assessment of haematological and clinical chemistry parameters. All animals were subjected to gross necropsy; weights of the main organs were recorded. A comprehensive range of tissues from all animals were examined histopathologically; additional sections of liver were investigated following staining with oil red O and Perl's stain.

One top-dose female was killed for humane reasons during week 16 following observations of dehydration, emaciation, faecal blood and vomiting. No further deaths occurred, and no additional signs of toxicity were observed. Body weight gain over the initial 2 weeks of dosing was reduced significantly in top-dose animals. Ophthalmoscopy did not reveal any treatment-related findings. Initial weight gains were lower in both sexes at the top dose level (Table 11). With the exception of the decedent female, feed consumption was unaffected by treatment. Variations in erythrocyte parameters appeared to be secondary to pre-test values. Higher total leukocyte counts were noted in top-dose females; values were significantly higher at weeks 13 and 26, largely attributable to elevated neutrophil counts. Similar increases were seen in mid-dose, but not high-dose, males (Table 11). Plasma albumin concentrations were significantly lower in both sexes at the top dose level, more consistently in males. There were also increases in ALP and aspartate aminotransferase (AST) activities and serum triglyceride concentrations in the top-dose groups (Table 11). Serum iron and total iron-binding capacity were reduced in females in a dose-related fashion (Table 11).

Mean absolute weights of adrenals and liver were higher in both sexes at the top dose level. Absolute kidney weights were significantly higher in top-dose females only. Mean ovary weight was higher in top-dose females; however, the value did not attain statistical significance. Gross necropsy revealed granular or swollen livers in top-dose males and pale livers in top-dose females.

Table 10. Findings in dogs receiving flutriafol for 90 days

Parameter	Males				Females			
	Dose (mg/kg bw per day)							
	0	1	5	15	0	1	5	15
Weight, week 13 (kg)	12.30	12.63	12.55	12.58	11.83	11.87	11.93	10.98
Weight gain (kg)								
- week 1	0.28	0.13	0.13	0.03*	0.15	0.10	0.05*	−0.05**
- week 2	0.43	0.15	0.18	0.18	0.18	0.13	0.15	−0.03
- week 13	1.83	1.48	1.20	1.48	1.85	1.83	1.58	0.95**
Haematology								
White blood cells (10^3/µl)								
- pre-test	12.03	11.78	13.43	12.98	13.88	13.15	14.28	12.80
- week 4	15.61	14.22	13.27	16.00	12.34	14.82	12.65	14.30
- week 13	13.11	15.78	12.95	19.02**	12.96	13.57	13.28	14.54
Clinical chemistry								
ALP (U/l)								
- pre-test	185	130	172	192	191	214	181	193
- week 4	189	136	192	269*	175	207	186	250*
- week 13	143	95	165	217*	127	165	128	231**
Triglycerides (mg/dl)								
- pre-test	41.5	36.5	44.5	41.8	38.5	38.3	47.0	35.3
- week 4	46.0	41.5	62.6**	49.0	39.7	47.0	34.9	49.2
- week 13	40.6	33.3	43.3	43.7	30.8	38.0	32.8	50.8**
Aminopyrine *N*-demethylase (µmol/h per gram)	13.8	13.1	27.1**	35.3**	15.2	15.6	25.1**	37.9**
Organ weights								
Liver (g)								
- absolute	395	414	416	466*	333	379	380	401
- adjusted for body weight	403	410	415	463**	321	370*	363	438**
Spleen (g)								
- absolute	59.7	63.0	38.2	75.1	42.4	47.5	53.3	53.7
- adjusted for body weight	61.8	61.8	37.8	74.5	41.3	46.7	51.7	57.2
Testes/ovaries (g)								
- absolute	21.33	22.00	22.23	22.23	1.010	0.844*	0.860*	0.720**
- adjusted for body weight	21.48	21.92	22.20	22.18	1.016	0.848*	0.869*	0.700**
Histopathology (*n* = 4)								
Liver: haemosiderin	1	0	0	3	0	0	0	4
Spleen: haemosiderin	0	0	0	3	0	0	0	0

From Kalinowski et al. (1982)
U, units; *$P < 0.05$; ** $P < 0.01$ (Student's *t*-test)

Microscopically, hepatotoxicity was characterized by the increased deposition of lipid in centrilobular hepatocytes in top-dose females; however, similar findings were not apparent in males (Table 12). The severity of haemosiderin pigmentation in liver sinusoidal cells and spleen was increased in both sexes at the top dose level. Increased vacuolation of the adrenal cortex zona glomerulosa was noted in all animals of both sexes at the top dose level and in one animal of each sex at 5 mg/kg bw per day (Table 12).

A NOAEL of 5 mg/kg bw per day can be determined for this study based on the effects on body weight, haematology, clinical chemistry, organ weights and pathology in both sexes at 20 mg/kg bw per day. The increased vacuolation of the zona glomerulosa of the adrenal cortex seen in both sexes at 5 mg/kg bw per day is not considered to be of clear toxicological significance because of the low incidence and severity. Although reduced serum iron concentrations were seen in all groups, values are variable, do not attain statistical significance and do not form a dose–response relationship (Stonard, 1988).

Table 11. Findings in dogs receiving flutriafol for 1 year

Parameter	Males				Females			
	Dose (mg/kg bw per day)							
	0	1	5	20	0	1	5	20
Body weight gain (kg)								
- week 1	0.38	0.27	0.25	0.00*	0.13	0.22	0.17	−0.17*
- week 2	0.60	0.45	0.45	0.25*	0.20	0.38	0.27	−0.02*
- week 52	3.52	2.80	3.19	3.08	2.75	2.82	2.25	2.28
White blood cells (10^3/µl)								
- pre-test	13.9	11.9	11.8	14.9	10.6	12.7	12.8	11.8
- week 4	12.2	13.9	14.7*	12.7	12.3	11.8	12.3	13.0
- week 13	14.3	14.8	16.3	14.3	13.6	13.2	12.9	17.6*
- week 26	14.0	14.9	16.7	16.1	12.9	13.2	16.1*	16.3*
- week 52	15.9	17.2	18.4	14.1	13.2	12.6	13.5	17.1
Neutrophils (10^3/µl)								
- pre-test	5.95	5.55	5.80	7.72	4.30	4.80	5.40	5.15
- week 4	6.88	6.99	6.54	6.35	5.15	6.66	6.33	6.95
- week 13	8.98	7.75	8.67	8.30	6.96	7.04	7.28	11.13*
- week 26	7.61	8.76	10.71*	10.19	6.75	8.21	8.84	9.41
- week 52	9.76	10.04	11.08	7.32	6.31	6.28	6.50	9.02
Lymphocytes (10^3/µl)								
- pre-test	5.82	4.30	3.82	4.15	4.50	6.07	5.17	5.00
- week 4	4.05	4.04	4.52	5.53*	4.64	4.10	4.51	4.50
- week 13	3.93	4.45	5.17	4.80	4.44	4.74	4.50	4.29
- week 26	4.80	4.69	4.54	4.39	4.51	3.71	5.35	5.50
- week 52	4.79	5.09	4.35	4.79	4.71	5.25	5.20	6.10
Albumin (g/dl)								
- pre-test	3.57	3.50	3.57	3.38	3.60	3.52	3.63	3.45
- week 4	3.61	3.45	3.59	3.07**	3.59	3.58	3.64	3.36
- week 13	3.75	3.70	3.65	3.03**	3.63	3.71	3.67	3.29**

Table 11 (continued)

Parameter	Males				Females			
	Dose (mg/kg bw per day)							
	0	1	5	20	0	1	5	20
- week 26	3.56	3.45	3.54	2.90**	3.66	3.70	3.56	3.30*
- week 52	3.72	3.63	3.77	3.02**	3.62	3.70	3.59	3.36
ALP (U/l)								
- pre-test	205	195	209	209	178	165	209	220
- week 4	173	180	187	277**	167	181	196	349**
- week 13	153	170	152	299	149	174	127	426**
- week 26	115	107	123	318**	93	96	134	271**
- week 52	116	127	151	328**	94	106	158	346**
Triglycerides (mg/dl)								
- pre-test	39.8	35.5	43.8	39.0	39.0	37.5	38.3	42.3
- week 4	33.4	36.1	42.6	41.9	37.8	40.5	42.3	61.2**
- week 13	23.1	30.3	25.2	27.9	26.4	27.3	27.3	45.2**
- week 26	33.5	35.6	28.6	27.2	34.0	34.2	28.0	37.0
- week 52	26.0	26.4	28.1	34.0*	35.7	34.1	35.4	43.8
Iron (mmol/l)								
- week 26	17.6	12.9	10.9	13.8	17.3	18.6	12.2	8.8
- week 52	12.8	33.4*	14.7	18.4	23.2	26.6	16.0	11.3
Total iron-binding capacity (mmol/l)								
- week 26	63.3	52.9	53.9	55.0	65.5	58.3	56.0	53.4*
- week 52	62.6	69.0	53.7	56.8	68.9	63.4	61.4	50.9

From Stonard (1988)
ALP, alkaline phosphatase; U, units; *$P < 0.05$; ** $P < 0.01$ (Student's *t*-test)

(b) Dermal application

Groups of Sprague-Dawley rats (10 of each sex per group) received dermal applications of flutriafol (purity 95.1%; batch No. UPL BX1) in PEG400 (dose volume 1 ml/kg bw) at 0, 250, 500 or 1000 mg/kg bw per day for 28 days. Routine observations included mortality, clinical signs and feed consumption. A functional observational battery (FOB) was performed on days 8, 14, 22 and 29, with motor activity assessments performed on day 29. At necropsy, blood samples were taken for clinical chemistry and haematological examinations; all animals received a gross examination, and an extensive range of tissues from control and top-dose rats was examined histopathologically. Dose levels were confirmed analytically.

There were no deaths or signs of systemic toxicity. Erythema and scabbing were present at the application site at the top dose level and in females from the 500 mg/kg bw per day group. Gross pathological and histopathological findings were similar in control and treated groups. Statistically significant changes in relative thymus weight in males and serum succinate dehydrogenase activity and cholesterol level in females are not considered adverse, as the values in test groups were within the control ranges.

The NOAEL for systemic toxicity is 1000 mg/kg bw per day, the highest dose tested. The NOAEL for local effects is 250 mg/kg bw per day, based on erythema and scabbing at 500 mg/kg bw per day (Barnett, 2007a).

Table 12. Organ weight and pathology findings in dogs receiving flutriafol for 1 year

Observation	Sex	Dose (mg/kg bw per day)			
		0	1	5	20
Organ weight (g)					
Adrenal	Male	1.73	1.67	1.71	2.02
	Female	1.68	1.61	1.84	2.31**
Liver	Male	452	475	477	574**
	Female	368	385	401	513**
Kidney	Male	66.6	68.0	62.5	67.3
	Female	55.7	53.5	58.3	64.8*
Ovary	Female	1.26	1.50	1.27	1.79
Histopathology (n = 4)[a]					
Adrenal: increased cortical zona glomerulosa vacuolation	Male	0	0	1 [1, 0, 0, 0]	4 [0, 4, 0, 0]
	Female	0	0	1 [1, 0, 0, 0]	4 [2, 2, 0, 0]
Liver: vacuolation	Male	0	0	0	0
	Female	0	0	0	1 [1, 0, 0, 0]
Liver: increased perivascular connective tissue	Male	0	0	0	1 [1, 0, 0, 0]
	Female	0	0	0	0
Liver: increased centrilobular hepatocyte lipid	Male	0	0	0	0
	Female	0	0	0	3 [1, 2, 0, 0]
Liver: sinusoidal cell haemosiderin pigmentation	Male	4 [1, 2, 1, 0]	4 [1, 2, 0, 1]	4 [2, 2, 0, 0]	4 [0, 0, 0, 4]
	Female	4 [1, 2, 1, 0]	4 [2, 1, 0, 1]	4 [1, 1, 2, 0]	4 [0, 0, 0, 4]
Spleen: haemosiderin pigmentation	Male	4 [1, 2, 1, 0]	4 [1, 2, 1, 0]	4 [0, 2, 2, 0]	4 [0, 0, 0, 4]
	Female	4 [1, 1, 2, 0]	4 [2, 1, 1, 0]	4 [1, 1, 2, 0]	4 [0, 0, 3, 1]

From Stonard (1988)

* $P < 0.05$; ** $P < 0.01$ (Student's t-test)

[a] Incidence [severity]. Laboratory severity scale: minimal, slight, moderate, marked.

(c) Exposure by inhalation

No data were submitted.

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Alderley Park (C57BL/10J/Alpk) mice (50 of each sex per group) were administered flutriafol (purity 93%; batch No. P10) in the diet at a level of 0 (two groups), 10, 50 or 200 ppm for 2 years. Routine observations and investigations were performed throughout the study. Blood smears (10 of each sex per group) were prepared following treatment for 12 and 18 months; a differential leukocyte count was performed, and erythrocyte morphology was investigated. Terminal blood samples were taken from all animals for the assessment of erythrocyte parameters. All animals were subjected to gross necropsy, and liver weights were recorded. A comprehensive list of tissues from all animals was investigated histopathologically.

Dietary analyses revealed the achieved concentrations, stability and homogeneity of the test material to be within acceptable limits. Achieved doses were 0, 1.2, 6.0 and 25 mg/kg bw per day in males and 0, 1.5, 7.4 and 30 mg/kg bw per day in females. A number of deaths occurred during the initial part of the study, apparently due to a failure to use the automatic water system effectively;

these animals were replaced. Overall survival was similar in all groups, was greater than 50% at 100 weeks and is considered to be acceptable for the assessment of carcinogenicity. The incidences of greying coat were increased in top-dose males; ocular discharge and thickened eyelids were observed with greater frequency in both sexes at the top dose level. Body weight and body weight gain were reduced at the top dose level (Table 13); a reduction at 50 ppm is not considered adverse, as it was transient in females and it was significant in males only at week 104, when mortality would have a marked impact on group means. Feed utilization efficiency was significantly reduced in top-dose males only (Table 13).

Examination of the blood smears prepared following treatment for 12 and 18 months did not reveal any effects of treatment. Investigation of terminal blood samples did not reveal any toxicologically significant effects on erythrocyte parameters. Significantly higher platelet and white blood cell counts were seen in top-dose males; white blood cell findings are attributable to significantly elevated neutrophil and lymphocyte counts. Similar findings were not apparent in females. There were no notable changes in blood parameters at 10 or 50 ppm.

Mean absolute and adjusted liver weights were significantly higher in both sexes at the top dose level; no other organs were weighed. Gross necropsy revealed slightly increased incidences of liver masses and discoloured and rough livers in top-dose males. Microscopically, liver toxicity was characterized by an increase in the incidence and severity of centrilobular fatty change and hepatocyte hypertrophy in both sexes at the top dose level (Table 13). The incidence and severity of centrilobular fatty change were also slightly increased in 50 ppm males. A marginally increased incidence of hepatocellular adenoma (4%) was observed in top-dose males; values are not statistically significant and are within the laboratory's historical control range (2, 0, 2, 0, 2, 0, 2, 0, 6, 0). Similar findings were not apparent in females, and the incidence of malignant hepatocellular tumours was unaffected by treatment in both sexes. The only non-hepatic finding of any note was a low incidence of vacuolation of white matter in the brain (Table 13); although this is a rare finding, it was not statistically significant, and there was no evidence of increased severity or a clear dose–response relationship.

No evidence of carcinogenicity was seen in this study; marginally increased incidences of benign hepatocellular tumours in males at the top dose level were within the historical control range. A NOAEL of 10 ppm (equal to 1.2 mg/kg bw per day) can be determined for this study, based on the increased incidence and severity of hepatic centrilobular fatty change in males at 50 ppm (equal to 6.0 mg/kg bw per day) (Hext, 1988).

Rats

Alpk:AP rats (64 of each sex per group) were administered flutriafol (purity 93%; batch No. P10) in the diet at a concentration of 0, 20, 200 or 2000 ppm for up to 104 weeks; 12 rats of each sex per group were sacrificed after treatment for 52 weeks. A standard range of observations and investigations was performed routinely during the study. All animals were subjected to gross necropsy; weights of the gonads, adrenals, kidneys, liver and brain were recorded. A comprehensive list of tissues from all animals was investigated histopathologically.

Dietary analyses showed the achieved concentrations, stability and homogeneity of the test material to be within acceptable limits. Achieved mean doses of flutriafol were 0, 1, 10 and 103 mg/kg bw per day and 0, 1.3, 13 and 130 mg/kg bw per day in males and females, respectively. A small number of accidental deaths occurred during blood sampling in the early part of the study. Mortality was comparable in all groups, and survival in all groups was above 50% at week 100. Survival in this study is considered to be adequate for the assessment of carcinogenicity. Clinical signs related to treatment were limited to increased incidences of rats appearing "thin" at the top dose level. Ophthalmoscopy revealed retinal pallor in both sexes at the top dose level, but was not investigated in the lower-dose groups (Table 14).

Cumulative weight gains by both sexes in top-dose animals were significantly lower throughout the study period. Reduced weight gain (~5%) in 200 ppm females at week 13 is not considered to

Table 13. Findings in mice receiving flutriafol for up to 2 years

Parameter	Males					Females				
	Dietary concentration (ppm)									
	0	0	10	50	200	0	0	10	50	200
Ocular discharge	60	25	52	69	172	63	101	96	93	240
Thickened eyelids	72	9	31	36	205	71	42	66	88	126
Survival, week 104 (%)	54	56	57	64	50	62	46	50	54	48
Body weight (g)										
- week 13	28.8	28.9	28.6	28.9	27.4**	23.7	23.6	23.7	23.2*	22.9**
- week 75	35.4	35.1	36.1	35.8	33.2**	28.7	28.3	29.6*	28.6	28.1
- week 104	34.0	33.2	33.2	32.2*	31.5**	28.1	27.7	27.8	28.0	26.4*
Weight gain (g)										
- week 12	8.7	9.1	8.4	9.2	7.3**	7.7	7.7	7.7	7.2*	7.2**
- week 52	13.8	14.2	13.7	13.7	12.0**	10.9	10.5	10.9	10.5	9.7**
Feed consumption, weeks 1–12 (g)	322.3	332.3	325.3	327.5	316.9*	360.3	361.3	356.3	350.7	348.6
Feed utilization, weeks 1–12 (g/100 g)	2.87	2.92	2.58	2.80	2.29**	2.14	2.13	2.30*	2.06	2.08
Liver weight (g)										
- absolute	1.67	1.65	1.63	1.68	2.19**	1.55	1.47	1.55	1.46	1.78**
- adjusted for body weight	1.64	1.64	1.61	1.70	2.24**	1.51	1.46	1.55	1.44	1.87**
Histopathology (n = 50)										
- liver fatty change	1	1	2	6	23	0	0	1	2	17
- liver centrilobular hypertrophy	1	0	1	1	14	0	0	0	1	3
- liver adenoma	1	0	1	1	2	0	1	0	0	0
- liver carcinoma	0	0	0	1	0	0	0	0	0	0
- brain, white matter vacuolation	0	0	0	1	2	0	0	0	1	1

From Hext (1988)
* $P < 0.05$; ** $P < 0.01$ (analysis of variance)

be of toxicological significance. Feed consumption and feed conversion efficiency were significantly lower throughout the study at the top dose level (Table 14). Haematology revealed statistically significant effects on erythrocyte parameters, consistent with mild microcytic anaemia, in both sexes at the top dose level. A tendency to higher total white blood cell and lymphocyte counts was seen in both sexes at the top dose level; findings were more marked in females (Table 14). Various clinical chemistry changes were seen at the top dose level: increases in total protein, cholesterol and total iron-binding capacity; and decreases in glucose, triglycerides and ALP (Table 14). Urinalysis revealed significantly lower urine volumes, higher specific gravity and lower pH in top-dose males.

At the interim sacrifice, mean adjusted kidney weights were significantly higher in females at and above 200 ppm, but only at the top dose level at terminal sacrifice; liver weights were significantly higher in both sexes at the top dose level. At terminal sacrifice, liver weights were significantly higher in both sexes at the top dose level and in males at 200 ppm (Table 15).

Gross necropsy of top-dose animals is reported to have found hepatic enlargement associated with discoloured foci. No further treatment-related findings were noted at gross necropsy. Histopathological investigation of the small number of animals dying prior to the interim sacrifice did not reveal any treatment-related findings. At the interim sacrifice, necropsy showed effects on the

Table 14. Findings in rats receiving flutriafol for 2 years

Parameter	Males				Females			
	Dietary concentration (ppm)							
	0	20	200	2000	0	20	200	2000
Body weight, week 104 (g)	625.4	621.6	613.4	549.0**	399.6	393.2	397.3	311.3**
Weight gain (g)								
- week 13	361.8	366.0	359.5	304.1**	161.4	158.8	153.5**	133.3**
- week 51	529.3	529.0	529.7	456.3**	240.6	233.3	232.3	175.9**
Feed consumption, weeks 1–13 (g)	2479	2503	2509	2270**	1841	1816	1825	1629**
Pale retina (n = 20)								
- week 52	0	nd	nd	3	0	nd	nd	3
- week 104	1	nd	nd	6	0	nd	nd	5
Haemoglobin (g/dl)								
- week 13	14.6	14.6	14.5	13.8**	14.6	14.2	14.5	13.8**
- week 26	15.8	15.8	15.9	15.1**	15.9	16.2*	16.0	14.9**
- week 52	16.4	16.5	16.4	15.6**	16.4	16.1	16.3	15.0**
Haematocrit (%)								
- week 13	46.3	45.9	46.3	44.1	47.3	46.5	47.4	45.1*
- week 26	47.9	47.9	48.1	46.3*	47.4	47.6	47.5	44.9**
- week 52	47.6	48.0	47.5	44.3**	46.3	45.1	45.8	42.3**
Mean corpuscular volume (fl)								
- week 13	50.3	49.7	49.3	49.0*	53.9	53.8	54.3	52.0**
- week 26	54.8	54.09	54.1	53.0**	58.8	58.8	59.4	55.3**
- week 52	55.3	56.0	54.7	52.5**	58.9	59.3	59.5	53.9**
Mean corpuscular haemoglobin (pg)								
- week 13	16.0	15.8	15.6	15.4	16.6	16.5	16.7	15.9*
- week 26	18.1	18.1	17.8	17.3**	19.7	20.0	20.1	18.4**
- week 52	19.1	19.3	18.8	18.5	20.9	21.1	21.2	19.1**
White blood cells (10^3/µl)								
- week 13	8.89	8.30	7.99	8.51	6.26	6.37	5.97	6.77
- week 26	7.38	6.89	7.53	8.02	4.87	4.66	4.50	5.71
- week 52	7.57	6.75	7.09	8.22	4.32	4.48	4.40	5.42*
Lymphocytes (10^3/µl)								
- week 13	7.12	6.78	6.54	6.90	5.02	5.41	4.87	5.57
- week 26	5.46	5.24	5.45	6.11	3.72	3.67	3.35	4.53*
- week 52	4.69	4.49	4.50	5.66*	2.97	3.20	2.79	4.02**
Total protein (g/dl)								
- week 13	7.13	7.18	7.22	7.52**	7.02	7.15	7.09	7.59**
- week 26	7.19	7.22	7.17	7.47*	7.32	7.28	7.38	7.89**
- week 52	7.41	7.36	7.24	7.42	7.63	7.79	7.72	8.17**
Glucose (mg/dl)								
- week 13	149	152	143	131**	143	140	145	137
- week 26	130	139*	133	130	134	129	136	126*
- week 52	131	127	123*	122*	132	128	131	128

Table 14 (continued)

Parameter	Males				Females			
	Dietary concentration (ppm)							
	0	20	200	2000	0	20	200	2000
Cholesterol (mg/dl)								
- week 13	74.3	76.3	74.0	67.8	72.4	77.8	75.6	95.8**
- week 26	85.3	86.3	85.3	79.2	82.1	83.4	82.2	106.3**
- week 52	116.3	135.1	126.3	108.6	98.2	104.2	99.8	137.2**
Triglycerides (mg/dl)								
- week 13	154	157	149	50**	90	98	106	97
- week 26	152	163	158	53**	126	108	136	114
- week 52	163	166	176	73**	171	186	181	152
ALP (U/l)								
- week 13	153	142	146	135*	95	90	87	82
- week 26	114	114	112	93**	60	58	60	51
- week 52	145	153	122*	100**	55	57	55	51
ALT (U/l)								
- week 13	48.3	51.9	50.1	74.3**	48.9	49.3	46.3	47.3
- week 26	58.6	58.3	54.3	64.0	53.1	58.0	60.8	54.6
- week 52	76.9	64..7	67.9	67.9	63.0	57.1	59.5	55.8
Serum iron (mmol/l), week 104	29.8	28.6	26.0	26.1	41.3	49.6	49.4	38.5
Total iron-binding capacity (mmol/l), week 104	86.4	67.5	73.0	92.8	76.3	88.8	88.4	107.0**

From Milburn et al. (1986)
ALP, alkaline phosphatase; ALT, alanine aminotransferase; nd, not determined; U, units; * $P < 0.05$; ** $P < 0.01$ (analysis of variance)

liver (centrilobular hepatocyte hypertrophy and fatty change) in both sexes at the top dose level and in 200 ppm males. In animals dying during the study and killed at termination, the target organs were liver, spleen and adrenals (Table 16). An apparently dose-related increase in the incidence of peripheral neuropathy (described as foci of degenerate nerve fibres) was seen in all treated groups of males dying during the study. However, the pattern is less marked when the total incidence (intercurrent deaths plus terminal sacrifice) is considered (Table 16), and it is not mirrored in females, indicating that it might be merely a variation in the time of observation of a common finding.

The overall pattern of tumours was consistent with that expected for the strain. Treatment-related neoplastic changes were seen in the liver, with slightly increased incidences of adenoma in both sexes at the top dose level and increased incidences of carcinoma in males at and above 200 ppm. Historical control data (Pigott, 1994) from 11 studies initiated in the same laboratory between July 1980 and March 1987 showed that the incidences in the flutriafol-treated animals are within the historical control range (Table 16). The incidence of Leydig cell tumours was also increased in this group; however, the incidence (10.9%) is reported to be within the laboratory's historical control range (2.8–10.9%; results from three contemporary studies); the absence of this common tumour in the concurrent control group is considered to be unusual. Incidences of mammary gland tumours were markedly lower in top-dose females, possibly secondary to the lower body weight in this group.

No evidence of carcinogenicity was seen in this study; slight increases in the incidence of liver adenoma and Leydig cell tumours in top-dose males are within the historical control ranges. An overall NOAEL of 20 ppm (equal to 1.0 and 1.3 mg/kg bw per day in males and females, respectively) can be determined for this study, based on the histopathological findings in the liver seen at 200 ppm (equal to 10 and 13 mg/kg bw per day in males and females, respectively) (Milburn et al., 1986).

Table 15. Organ weights in rats receiving flutriafol for up to 2 years

Organ	Organ weights (g)							
	Males				Females			
	Dietary concentration (ppm)							
	0	20	200	2000	0	20	200	2000
Interim sacrifice								
Kidneys								
- absolute	3.65	3.61	3.72	3.46	2.20	2.18	2.39*	2.26
- adjusted[a]	3.51	3.55	3.65	3.79	2.13	2.16	2.31**	2.42**
Liver								
- absolute	21.8	21.0	23.9	26.6**	13.1	12.6	13.6	14.5*
- adjusted[a]	20.9	20.6	23.4	28.7**	12.5	12.5	12.9	16.0**
Terminal sacrifice								
Kidneys								
- absolute	4.21	4.43	4.58	4.61	2.84	2.80	2.71	2.77
- adjusted[a]	4.23	4.45	4.59	4.57	2.70	2.71	2.62	3.01*
Liver								
- absolute	19.8	20.9	22.0**	25.7**	14.5	13.9	13.7	15.6
- adjusted[a]	19.5	20.7	21.9**	26.2**	13.6	13.3	13.1	17.3**

From Milburn et al. (1986)
* $P < 0.05$; ** $P < 0.01$ (analysis of variance)
[a] Organ weights adjusted for terminal body weight by analysis of covariance.

2.4 Genotoxicity

Testing of the genotoxicity of flutriafol has been performed in a range of assays (Table 17). Equivocal results were seen at cytotoxic concentrations in two reverse mutation assays with mouse lymphoma L5178Y cells. The remaining in vitro and all the in vivo tests were negative. The overall database is considered adequate to conclude that flutriafol is unlikely to be genotoxic.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

Alpk:AP rats (F_0 generation; 15 males, 30 females per group) were administered flutriafol (purity 93%; batch No. P10) in the diet at a concentration of 0, 60, 240 or 1000 ppm for 12 weeks prior to mating to produce F_{1A} litters. Two females were housed with one male until mating or for a maximum of 10 days. If mating was unsuccessful, the male was removed and replaced with an animal of proven fertility. Litters were not standardized at day 4, and the F_{1A} pups were weaned at day 29. A week after weaning, F_0 parents were remated to produce F_{1B} litters; litters were weaned at day 29, but remained housed by litter until selection at day 36. Selected F_{1B} pups (15 males, 30 females per group) were administered the test material for 11 weeks prior to mating to produce the F_{2A} litters. Litters were not standardized, and the F_{2A} pups were weaned at day 29. A week after weaning, the F_1 parents were remated to produce F_{2B} litters.

All rats were observed daily for clinical and behavioural abnormalities; more detailed physical examinations were carried out weekly. F_0 and F_1 parents were subjected to gross necropsy; weights of the testes, ovaries and liver were recorded. Testes, epididymides, prostate, seminal vesicles, ovaries,

Table 16. Histopathology findings in rats receiving flutriafol for up to 2 years

Parameter	Incidence of finding							
	Males				Females			
	Dietary concentration (ppm)							
	0	20	200	2000	0	20	200	2000
Interim sacrifice								
Number of animals	*11*	*11*	*12*	*11*	*12*	*12*	*12*	*12*
Liver: centrilobular hypertrophy	0	0	1	10*	0	0	0	4*
Liver: fatty change	0	0	3	11*	0	0	0	1
Liver: haemosiderin accumulation	0	0	0	0	0	0	0	12*
Spleen: haemosiderin accumulation[a]	11 (1.1)	11 (1.5)	12 (1.7)	10 (1.7)	12 (1.8)	12 (2.0)	12 (2.0)	12 (3.0)
Intercurrent deaths								
Number of animals	*26*	*25*	*24*	*26*	*33*	*28*	*27*	*22*
Liver: centrilobular hypertrophy	0	0	0	2	0	0	0	1
Liver: fatty change	4	3	13*	24*	10	12	9	15
Liver: spongiosis	1	1	5	1	0	0	0	0
Liver: clear cell focus	1	1	2	3	0	1	0	0
Liver: haemosiderin accumulation	0	0	0	0	0	0	0	10*
Adrenal: macrophages	0	0	1	4	4	1	4	15*
Adrenal: vacuolation	4	3	9	12*	1	0	1	2
Thyroid: cystic foci	0	0	0	3	0	0	0	0
Spleen: increased haemosiderin	0	0	0	9*	9	3	7	18*
Peripheral neuropathy	2	4	10*	16*	10	5	8	7
Kidney: nephropathy	21	20	21	24	28	23	21	20
Seminal vesicle: atrophy	1	1	3	4	—	—	—	—
Terminal sacrifice								
Number of animals	*23*	*23*	*26*	*24*	*19*	*23*	*25*	*29*
Liver: centrilobular hypertrophy	0	0	0	1	0	0	0	3
Liver: fatty change	8	9	14	24*	2	3	6	18*
Liver: clear cell focus	8	12	23*	17*	2	1	1	0
Liver: haemosiderin accumulation	0	1	1	0	0	0	0	18*
Adrenal: macrophages	0	1	0	1	9	7	8	25*
Adrenal: vacuolation	17	14	21	12	4	1	3	2
Thyroid: cystic foci	0	0	1	2	1	1	0	1
Spleen: increased haemosiderin	0	0	1	2	1	0	2	25*
Peripheral neuropathy	17	18	18	13	15	16	9	17
Kidney: nephropathy	22	22	24	24	19	19	23	22
Total neoplastic								
Number of animals	*64*	*64*	*64*	*64*	*64*	*64*	*64*	*64*
Liver: adenoma (HC 0–6.7%)	0	0	0	1	0	0	0	2
Liver: carcinoma (HC 0–5.8%)	0	0	1	2	0	0	0	0
Leydig cell tumour (HC 2.8–10.9%)	0	4	3	7	—	—	—	—
Mammary: adenocarcinoma	—	—	—	—	6	4	5	2
Mammary: fibroadenoma	—	—	—	—	4	7	6	1
Total peripheral neuropathy (*n* = 52)	19	22	28	29*	25	21	17	24

HC, historical control range (11 studies 1980–1987); * $P < 0.05$

[a] Incidence (mean severity score).

Table 17. Results of studies of genotoxicity with flutriafol[a]

End-point	Test object	Concentration	Purity (%); batch	Result	Reference
In vitro					
Reverse mutation	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537; *Escherichia coli* WP2*uvrA*	3–5000 µg/plate, ±S9 (plate incorporation) 33–5000 µg/plate, ±S9 (pre-incubation)	95.1; UPL BX-1	Negative	Sokolowski (2006)
Reverse mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538	1.6–5000 µg/plate, ±S9	93; P10	Negative	Callander (1988)
Reverse mutation	Mouse lymphoma L5178Y cells	12.5–600 µg/plate, −S9 25–600 µg/plate, +S9	95.1; UPL BX-1	Equivocal ±S9 with cytotoxicity	Wollny (2006)
Reverse mutation	Mouse lymphoma L5178Y cells	50–800 µg/plate, −S9 150–1500 µg/plate, +S9	Not reported	Equivocal +S9 with cytotoxicity Negative –S9	McGregor & Riach (1984)
Chromosomal aberration	Human lymphocytes	91–1514 µg/ml, +S9 92–1250 µg/ml, −S9	95.1; UPL BX-1	Negative	Kunz (2007)
Chromosomal aberration	Human lymphocytes	25, 125, 250 µg/ml, ±S9	92.8; batch not reported	Negative	Howard (1989)
In vivo					
Chromosomal aberration	Alpk rats	0, 15, 75 or 150 mg/kg bw (gavage) single or 5 consecutive days	93; batch not reported	Negative	Richardson et al. (1982)
Bone marrow micronuclei	Alpk mice	0, 94 or 150 mg/kg bw (gavage) single	93; P10	Negative	Sheldon et al. (1986)
Liver unscheduled DNA synthesis	Alpk rats	0, 250, 500 or 1000 mg/kg bw (gavage) single	93; P10	Negative	Trueman (1987)

DNA; deoxyribonucleic acid; S9, 9000 × *g* supernatant from livers of rats

[a] Vehicle dimethyl sulfoxide for in vitro studies, corn oil for in vivo studies.

uterus and cervix of control and top-dose animals were investigated histopathologically; investigations in intermediate-dose groups were limited to the liver and grossly abnormal tissues. Clinically abnormal F_{1A} and F_{2A} pups were subject to gross necropsy at day 36 postpartum; the remainder were discarded. Non-selected F_{1B} (5 of each sex per group) and F_{2B} pups (10 of each sex per group) were subject to gross necropsy at day 36. Testes, epididymides, prostate, seminal vesicles, ovaries, uterus and cervix from control and top-dose animals were investigated histopathologically; investigations in intermediate-dose groups were limited to the liver and grossly abnormal tissues. Pups found dead at up to and including 18 days of age were subjected to gross necropsy. Those pups found dead at over 18 days were subjected to gross necropsy; the testes, epididymides, prostate, seminal vesicles, ovaries, uterus, cervix, liver and grossly abnormal tissues were examined histopathologically.

Some variation was evident between the numerical information in the main study report, the individual animal data and a supplementary analysis (Plassmann & Hofer, 2006). The variations were relatively minor and do not have an impact on the overall evaluation.

Dietary analyses revealed the stability, homogeneity and achieved concentrations of the test material to be within acceptable limits. Achieved doses were 0, 3.5, 14 and 56 mg/kg bw per day.

Parental animals. No treatment-related deaths occurred, and no signs of toxicity were observed. Body weight gains and feed consumption were consistently lower in the top-dose groups during most

phases of the study (Table 18). Liver weights of both sexes at the top dose level were significantly higher; histopathology revealed increased incidences of hepatic centrilobular hypertrophy in top-dose males and fatty change in both sexes at the top dose and in 240 ppm F_1 males. Mean testes weight (adjusted for body weight) was lower in top-dose F_1 males; mean ovary weight was increased in F_1 females (Table 18). Mating indices were comparable in all groups; however, the fertility index at the top dose level was lower for the F_{1A} litter; a similar finding was not apparent for the F_{1B} litter or F_2 litters (Table 19).

Offspring. The number of F_{1A} litters at the top dose level was lower as a result of the reduced fertility index in this group. Mean litter size was lower at the top dose level in all matings (Table 20). Pup birth weights, weight gains and the number of postnatal deaths were comparable in all groups. Necropsy of the F_{1B} and F_{2B} litters revealed evidence of hepatic fatty change at the top dose level.

A NOAEL for reproductive toxicity of 240 ppm (equal to 14 mg/kg bw per day) can be determined for this study, based on the consistently lower litter sizes in all generations at the top dose level and the lower fertility index in F_0 parents seen for the first generation.

A NOAEL for parental toxicity of 60 ppm (equal to 3.5 mg/kg bw per day) can be determined, based on the liver histopathology (fatty change) in F_1 males at 240 ppm.

A NOAEL for offspring toxicity of 240 ppm (equal to 14 mg/kg bw per day) can be determined, based on the liver histopathology (fatty change) at the top dose level (Tinston et al., 1986).

In a screening reproductive toxicity study, flutriafol (purity 95.1%; batch No. UPL BX 1) was administered to groups (five of each sex) of Han Wistar rats at a concentration of 0, 30, 60, 240 or 1000 ppm in the diet. The males were treated for 70 days and the females for 14 days prior to pairing (1:1). The resultant offspring were culled to four of each sex per litter at day 4, then reared to weaning, separated from the mother and reared for a further 2 weeks on their respective diets until necropsy. Regular assessments were made of clinical signs, body weights and feed consumption. Routine examinations were made of mating performance and gestation, parturition and lactation status. Age and body weight at vaginal opening and preputial separation were determined. Limited gross and microscopic examinations were performed, primarily of reproductive organs. Sperm analyses (morphology, motility and count) were performed on parental males from control and top-dose groups.

Table 18. Parental and offspring findings in rats receiving flutriafol

Parameter		Dietary concentration (ppm)							
		0	60	240	1000	0	60	240	1000
		Males				**Females**			
F_0	Pre-mating weight (g)	482.2	467.1	465.2	454.3	271.9	270.9	269.7	260.0
	Weight gain (g)	411.4	397.1	394.9	384.6	207.3	206.2	205.3	195.0
	Feed consumption (g)	2451	2381	2388	2326**	1669	1669	1664	1609**
	Feed utilization (g feed/g weight gain)	5.98	6.04	6.06	6.06	8.12	8.11	8.11	8.17
F_1	Pre-mating weight (g)	469.7	476.3	488.1	473.1	270.7	272.5	265.5	260.9
	Weight gain (g)	354.4	364.0	367.5	349.9	167.3	167.3	161.0	150.0
	Feed consumption (g)	2286	2351	2377	2355	1594	1631	1575	1566
	Feed utilization (g feed/g weight gain)	6.47	6.49	6.53	6.78	9.67	9.77	9.79	10.49**

Table 18 (continued)

Parameter		Dietary concentration (ppm)							
		0	60	240	1000	0	60	240	1000
		F_{1A}				**F_{1B}**			
F_0	Weight, day 0 (g)	274.1	272.5	277.6	260.3*	310.9	314.0	312.4	300.5*
	Weight gain, day 8 (g)	26.5	28.4	25.9	22.9	23.8	22.5	22.9	19.5*
	Weight gain, day 15 (g)	57.7	61.5	56.6	49.4**	52.5	50.8	50.9	42.8**
	Weight gain, day 22 (g)	134.9	138.1	134.3	121.9	121.4	127.5	120.1	106.8**
	Weight, day 22 (g)	409.0	410.6	411.9	382.2	432.3	441.5	432.5	407.3
		F_{2A}				**F_{2B}**			
F_1	Weight, day 0 (g)	287.0	284.1	282.4	276.5	312.8	319.4	309.0	304.4
	Weight gain, day 8 (g)	23.6	22.8	24.4	23.1	23.7	21.5	22.7	17.8**
	Weight gain, day 15 (g)	50.2	51.3	53.5	50.3	52.8	51.5	52.1	46.2**
	Weight gain, day 22 (g)	114.0	110.0	117.6	109.1	121.8	130.1	123.3	115.8
	Weight, day 22 (g)	401.0	394.1	399.7	385.6	434.6	449.5	432.3	420.2
Organ weights (g)		**Males**				**Females**			
F_0	Liver, absolute	21.3	20.8	21.9	25.7**	14.3	13.8	13.8	15.2
	Liver, adjusted	21.0	21.4	22.1	25.7**	13.6	13.8	13.8	16.0**
F_1	Liver, absolute	21.7	22.9	22.7	28.1**	12.6	13.1	12.9	14.0**
	Liver, adjusted	22.0	22.8	22.6	28.0**	12.6	12.8	12.9	14.4**
F_0	Testes/ovary, absolute	3.61	3.70	3.78	3.66	0.187	0.177	0.186	0.187
	Testes/ovary, adjusted	3.61	3.71	3.78	3.66	0.186	0.177	0.186	0.188
F_1	Testes/ovary, absolute	3.80	3.66	3.88	3.62	0.201	0.188	0.200	0.238
	Testes/ovary, adjusted	3.82	3.65	3.88	3.61*	0.201	0.190	0.200	0.235
Liver histopathology		**Males**				**Females**			
F_0	Centrilobular hypertrophy	0/15	0/15	0/15	2/15	0/30	0/30	0/30	0/30
F_1		0/15	0/15	0/15	4/15	0/30	0/30	0/30	0/30
F_0	Fatty change	0/15	1/15	0/15	8/15*	1/30	1/30	0/30	5/30
F_1		0/15	0/15	5/15*	13/15*	0/30	0/30	0/30	3/30

From Tinston et al. (1986)
* $P < 0.05$; ** $P < 0.01$

Table 19. Reproductive findings in rats receiving flutriafol

Parameter		First litter				Second litter			
		Dietary concentration (ppm)							
		0	60	240	1000	0	60	240	1000
		Males							
Mating index (%)	F_0	93	90	87	87	75	76	82	88
	F_1	87	87	93	87	86	76	80	85
Fertility index (%)	F_0	78	93	77	54	93	84	84	83
	F_1	73	85	86	77	87	90	96	97
		Females							
Mating index (%)	F_0	93	90	87	87	97	93	97	97
	F_1	87	87	93	87	93	93	93	97

Table 19 (continued)

Parameter		First litter				Second litter			
		Dietary concentration (ppm)							
		0	60	240	1000	0	60	240	1000
Fertility index (%)	F_0	78	93	77	54	100	93	90	93
	F_1	73	85	86	77	96	93	96	100
		F_{1A}				**F_{1B}**			
Litters (no.)	F_0	21	25	20	14	26	26	26	26
- with live pups		21	25	20	14	26	26	26	26
- all pups alive		16	22	15	14	24	24	21	22
- with dead pups		5	3	5	0	2	2	5	4
- all pups dead		0	0	0	0	0	0	0	0
		F_{2A}				**F_{2B}**			
Litters (no.)	F_1	18	22	24	20	26	26	27	29
- with live pups		18	22	24	20	26	26	26	28
- all pups alive		18	20	23	15	24	23	25	19
- with dead pups		0	0	0	0	0	0	1	1
- all pups dead		0	2	1	5	2	3	1	9

From Tinston et al. (1986)

Achieved mean doses were 0, 1.6, 3.3, 13 and 54 mg/kg bw per day in the male groups and 0, 2.3, 4.3, 18 and 73 mg/kg bw per day in the female groups. There were no deaths or treatment-related clinical signs and no effects on sperm parameters. At 1000 ppm, a number of effects were produced: reduced feed consumption and body weight gain in females during the pre-pairing, gestation and lactation periods; increased duration of gestation; decreased implantation numbers; increased implantation loss (partly due to increased number of pups born dead); decreased litter size; increased number of postnatal losses; increased liver weights; decreased pup weights from birth to weaning and thereafter; and delayed vaginal opening (possibly secondary to reduced delayed body weight). Hepatic fatty change and hepatocellular hypertrophy were seen in both sexes; ovarian stromal cell hyperplasia incidence and severity were slightly increased.

At 240 ppm (equal to 13 mg/kg bw per day), effects included increased post-implantation loss (to 13%); slightly decreased birth index; and hepatic fatty change and hepatocellular hypertrophy.

The NOAEL for parental, pup and reproductive effects was 60 ppm (approximately 4 mg/kg bw per day) (Gerspach, 2008a).

Groups of Han Wistar rats (24 of each sex) were exposed to flutriafol (purity 95.1%; batch No. UPL BX 1) at a concentration of 0, 30, 80, 150 or 300 ppm in the diet. Parental animals were exposed for 10 weeks before pairing and through gestation, lactation, weaning and selection of the F_2 parental generation through to weaning of the F_2 pups. Litters were culled to four of each sex on day 4. One pup of each sex per litter was given a gross and histopathological examination. All F_2 parental animals received a gross pathological and histopathological examination. Regular assessments were made of clinical signs, body weights and feed consumption. Routine examinations were made of mating performance and gestation, parturition and lactation status. Age and body weight at vaginal opening and preputial separation were determined. Sperm analyses (morphology, motility and count) were performed on parental males from control and top-dose groups.

Achieved doses were 0, 1.5, 4.0, 7.4 and 16 mg/kg bw per day in the male groups and 0, 2.0, 5.3, 9.7 and 21 mg/kg bw per day in the female groups. Findings seen in the screening study at

Table 20. Offspring findings for rats receiving flutriafol

Parameter	Time	Dietary concentration (ppm)			
		0	60	240	1000
F_{1A}					
Deaths	Days 1–29	9	10	18	6
Litter size (no.)	Day 1	11.7	11.6	12.2	11.2
	Day 29	11.2	11.2	11.3	10.8
Weight, M, F (g)	Day 1	6.1, 5.8	6.3, 5.9	6.1, 5.9	6.5, 6.2
Weight gain, M, F (g)	Day 5	3.2, 3.2	3.4, 3.5	2.9, 2.7	3.8, 3.6
	Day 29	71.8, 67.1	72.4, 68.5	69.0, 62.7	70.1, 64.6
F_{1B}					
Deaths	Days 1–29	23	15	25	21
Litter size (no.)	Day 1	11.3	11.9	10.8	9.7
	Day 29	10.4	11.3	9.9	8.9
Weight, M, F (g)	Day 1	6.2, 5.9	6.1, 5.7	6.2, 5.9	6.3, 5.9
Weight gain, M, F (g)	Day 5	3.2, 3.2	3.4, 3.3	3.4, 3.1	4.0*, 4.0**
	Day 29	73.8, 68.9	73.5, 67.8	74.1, 67.2	75.4, 70.4
F_{2A}					
Deaths	Days 1–29	10	8	7	4
Litter size (no.)	Day 1	12.0	11.2	10.8	9.5
	Day 29	11.4	10.8	10.5	9.3
Weight, M, F (g)	Day 1	6.4, 6.1	6.2, 5.8	6.6, 6.1	6.6, 6.2
Weight gain, M, F (g)	Day 5	3.5, 3.5	3.7, 3.7	3.7, 3.6	4.2, 4.0
	Day 29	70.6, 66.3	74.0, 69.9	74.9, 68.9	73.9, 68.6
F_{2B}					
Deaths	Days 1–29	18	12	4	11
Litter size (no.)	Day 1	10.7	12.0	9.9	9.4
	Day 29	10.0	11.6	9.7	9.3
Weight, M, F (g)	Day 1	6.3, 6.0	6.4, 6.0	6.6, 6.2	6.5, 6.1
Weight gain, M, F (g)	Day 5	3.6, 3.3	3.9, 3.8	3.8, 3.7	4.0, 3.8
	Day 29	74.9, 68.6	77.7, 71.3	75.8, 71.4	76.2, 69.7
Liver: fatty change, M, F					
F_{1A}		0/0, 0/1	0/2, 0/1	0/3, 0/1	0/2, 0/2
F_{1B}		0/6, 0/8	0/5, 0/6	0/5, 0/6	1/7, 1/6
F_{2B}		0/10, 0/10	0/10, 0/10	0/10, 0/11	5/10, 1/10

From Tinston et al. (1986)
F, female; M, male; * $P < 0.05$; ** $P < 0.01$

240 ppm were not repeated in this study at 300 ppm. There were no deaths or clinical signs of toxicity. Feed consumption and body weights were similar in all groups. There were no adverse effects on mating, estrous cycling, sperm parameters, gestation length, birth or litter parameters in the F_1 or F_2 generations (Table 21). The time to sexual maturation was slightly but statistically significantly increased in F_1 offspring of both sexes (Table 21), but the values were within the historical control range (26.7–28.3 days in males and 33.0–35.1 days in females), and this is not considered to represent an adverse effect. Post-implantation losses were slightly increased at the top dose level in both generations but had an insignificant effect on litter size (Table 21) and are not considered to be adverse.

Table 21. Reproductive findings in rats dosed with flutriafol

	Dietary concentration (ppm)				
	0	30	80	150	300
F_1 generation					
Post-implantation loss (%)	8.8	9.1	8.2	9.9	10.6
Litter size, day 1	12.2	12.3	11.6	12.0	12.1
Litter size, day 4 post-cull	8.0	8.0	7.9	7.7	7.7
Litter size, day 21	8.0	8.0	7.9	7.5	7.7
Body weight, day 21, M/F (g)	50/48	51/49	50/48	51/49	51/49
Preputial separation (day)	27.1 ± 0.8	27.2	27.5	27.6	27.9 ± 0.9*
Preputial separation (weight; g)	79 ± 6	—	—	—	84 ± 8
Vaginal patency (day)	33.7 ± 1.5	33.5	34.4	33.6	34.6 ± 1.8
Vaginal patency (weight; g)	108 ± 9	—	—	—	113 ± 11
F_2 generation					
Post-implantation loss (%)	8.3	7.5	8.3	9.3	10.7
Litter size, day 1	12.6	12.3	12.4	11.8	11.9
Litter size, day 4 post-cull	8.0	7.8	8.0	7.4	7.9
Litter size, day 21	8.0	7.8	8.0	7.4	7.9
Body weight, day 21, M/F (g)	52/51	52/51	52/51	52/51	52/51

From Gerspach, Flade & Chevalier (2009)
F, female; M, male; * $P < 0.05$

Liver weights were increased slightly (< 10%) at 300 ppm in parental animals of both sexes in both generations. The increased liver weight was associated with centrilobular hepatocyte hypertrophy. A parental NOAEL of 150 ppm (equal to 7.4 mg/kg bw per day) can be determined, based on the liver histopathology (fatty change) in F_1 males at 300 ppm.

There were no alterations in organ weights or histopathological findings in the reproductive organs examined.

The NOAEL for reproductive and pup toxicity is 300 ppm (equal to 16 mg/kg bw per day), the highest dose tested in this study (Gerspach, Flade & Chevalier, 2009). Some effects were observed at the highest dose level (e.g. the time to sexual maturation was slightly but statistically significantly increased in F_1 offspring of both sexes, and post-implantation losses were slightly increased at the top dose level in both generations), but these were marginal and considered to be of no biological significance.

(b) Developmental toxicity

Rats

Mated female Alderley Park rats (24 per group) were gavaged with flutriafol (purity 93%; batch No. P10) suspended in corn oil at 0, 10, 50 or 125 mg/kg bw per day on gestation days 6–15. Animals were observed daily for clinical signs; body weights and feed consumption were measured at intervals during the study. Dams were sacrificed and the uterine contents investigated on day 21. All fetuses were weighed and examined for external abnormalities. Approximately two thirds of the fetuses were investigated for internal abnormalities prior to evisceration and staining with alizarin red for the assessment of skeletal findings. The remaining fetuses were investigated by serial sectioning (heads) and dissection (viscera).

No deaths occurred during the study period. Signs of toxicity were limited to genital/ventral staining in 16 animals at the top dose level. Body weight gains and feed consumption at the top

dose level were significantly lower than those of controls over the dosing period and post-treatment. The numbers of corpora lutea and implantations were comparable in all groups; however, mean litter size at the top dose level was significantly lower than that of controls as a result of significantly higher post-implantation loss (Table 22). Mean gravid uterus weights, litter weights and fetal weights were also significantly lower at the top dose level; similar effects were not apparent in other treated groups.

The total incidences of external and visceral defects were unaffected by treatment; incidences of individual findings were comparable in all groups (Table 23). Three fetuses had major skeletal defects: at 50 mg/kg bw per day, a single fetus had multiple skeletal defects (missing vertebral arches); at 125 mg/kg bw per day, two fetuses had pronounced kinked ribs. The total incidence of minor skeletal defects was significantly increased (in a dose-related fashion) in all treated groups (Table 23). A number of individual findings were significantly increased in incidence at the top dose level, and a smaller number were significantly increased at 50 mg/kg bw per day. At 10 mg/kg bw per day, a small number of individual findings were increased in incidence, including partially ossified occipital, unossified odontoid, increased fontanelle size and unossified calcaneum (Table 23). Mean scores for the degree of manus and pes ossification were significantly higher in all treated groups (in a dose-related fashion), indicating delayed or reduced ossification. Although some of the findings at the lowest dose level were within the range of contemporary historical control data (Moxon, 2005a), the findings for odontoid and occipital ossification and manus and pes scores were outside the historical control range (Table 23).

No evidence of teratogenicity was seen in this study; litter size was reduced at the top dose level as a result of increased post-implantation loss associated with maternal toxicity. A maternal NOAEL of 50 mg/kg bw per day can be determined for this study, based on the clinical signs and body weight effects at the top dose level. Minor body weight effects at 50 mg/kg bw per day are not considered to be toxicologically significant. A developmental NOAEL cannot be determined for this study as a result of the increased incidences of a number of fetal skeletal parameters mainly indicative of reduced or retarded ossification at the lowest dose level (Killick et al., 1982).

In an initial range-finding developmental toxicity study, female Han Wistar rats (five per group) were administered flutriafol (purity 95.1%; batch No. UPL BX 1) by gavage at 0 (corn oil), 5, 10, 50 or 150 mg/kg bw per day from gestation days 6 to 20. The dams were sacrificed on gestation day 21, and the fetuses were removed by caesarean section. Fetuses were examined for external anomalies, and skeletons from all groups except 150 mg/kg bw per day were stained with alizarin red and alcian blue.

One top-dose animal was sacrificed on day 12. Treatment at 150 mg/kg bw per day resulted in the occurrence of behavioural changes or clinical signs and decreased feed consumption and strongly retarded body weight from the first day of dosing; this was considered to be mainly due to embryonic/fetal resorptions in all pregnant animals in this group. There were only two dams with only three live fetuses in which weights were also markedly low. Skeletal and cartilage examinations were not performed for this group. At 50 mg/kg bw per day, slightly reduced maternal feed consumption and retarded body weight development occurred during the treatment period.

The number of skeletal variations (rudimentary cervical ribs and supernumerary rudimentary thoracic ribs) was increased in fetuses from all treated groups. Increased incidences of branched xiphoid cartilage were noted at 50 mg/kg bw per day. One fetus at 150 mg/kg bw per day had multiple external abnormalities (a domed head, ablepharia, micrognathia and a suspected cleft palate).

This preliminary study failed to show a fetal NOAEL as a result of increases in thoracic and cervical ribs at 5 mg/kg bw per day and above. The maternal NOAEL was 10 mg/kg bw per day, based on reduced body weight gain. Within the limited study design, there was no indication of teratogenicity in the absence of severe maternal toxicity (Gerspach, 2008b).

Table 22. Maternal and litter findings in rats administered flutriafol during pregnancy

Parameter	Dose (mg/kg bw per day)			
	0	10	50	125
Maternal findings				
Body weight (g)				
- day 6	284.4	282.6	286.0	272.8
- day 15	326.0	326.2	327.4	303.6
- day 21	415.2	412.3	412.8	363.8
Weight gain (g) [% of control]				
- days 0–6	29.1	31.1	30.8	31.7
	[—]	[107%]	[106%]	[109%]
- days 6–9	6.4	10.4	6.0	3.0
	[—]	[163%]	[93.8%]	[46.9%]
- days 6–15	41.6	43.6	41.4	30.8*
	[—]	[105%]	[100%]	[74.0%]
- days 15–21	89.2	86.1	85.4	60.2*
	[—]	[96.5%]	[95.7%]	[67.5%]
Feed consumption (g/day) [% of control]				
- days 6–15	22.6	22.4	22.2	18.8**
	[—]	[99.1%]	[98.2%]	[83.2%]
- days 15–21	35.5	35.0	35.4	30.6**
	[—]	[98.6%]	[99.7%]	[86.2%]
Litter findings				
Mated (no.)	24	24	24	24
Litters (no.)	20	21	23	21
Corpora lutea (no.)	15	14.4	13.8	14.0
Implantations (no.)	12.9	12.6	11.8	12.4
Early intrauterine deaths [% of total]	15 [6.3%]	19 [7.2%]	16 [6.1%]	40 [14.8%]
- litters affected	6/20	13/21*	10/23	14/21*
Late intrauterine deaths [% of total]	0	2 [0.7%]	1 [0.4%]	46 [18.7%]
- litters affected	0/20	2/21	1/23	14/21**
Pre-implantation loss (%)	13.5	10.3	13.8	12.2
- litters affected	15/20	10/21	12/23	13/21
Post-implantation loss (%)	6.3	7.9	6.4	33.5
- litters affected	6/20	13/21*	10/23	17/21**
Litter size (no.)	12.2	11.7	11.1	8.4*
Gravid uterus weight (g)	84.4	81.7	79.2	61.5**
Litter weight (g)	63.9	59.9	57.8	38.7**
Fetal weight (g)	5.27	5.09	5.24	4.44**

From Killick et al. (1982)
* $P < 0.05$; ** $P < 0.01$ (*t*-test or Fisher's test)

Table 23. Fetal findings for rats administered flutriafol during pregnancy

Parameter	Historical control range[a] (%)	Dose (mg/kg bw per day)							
		0		10		50		125	
Total findings [fetal incidence]									
Minor external/visceral defects: total	—	6	[2.5%]	12	[5.0%]	9	[3.7%]	9	[5.3%]
Major external/visceral defects: total	—	—	—	1	[0.4%]	1	[0.4%]	1	[0.6%]
Minor skeletal defects: total	—	65	[40.6%]	92**	[55.4%]	109**	[64.9%]	101**	[88.6%]
Major skeletal defects: total	—	—	—	—	—	1	[0.6%]	2	[1.8%]
Skeletal findings [fetal incidence]									
Occipital: partially ossified	1–39	132	[82.5%]	159**	[95.8%]	166**	[98.8%]	114**	[100%]
Fontanelle: increased	0–3.6	—	—	2	[1.2%]	3	[1.8%]	16**	[14.0%]
Odontoid: not ossified	4–10	14	[8.8%]	29*	[17.5%]	42**	[25%]	64**	[56.1%]
Cervical arches 3–6 partially ossified	—	—	—	—	—	—	—	7**	[6.1%]
Cervical rib									
- unilateral	0–13	4	[2.5%]	6	[3.6%]	16**	[9.5%]	21**	[18.4%]
- bilateral	0–1	1	[0.6%]	3	[1.8%]	10**	[6.0%]	20**	[17.5%]
- unilateral/bilateral	0–14	5	[3.1%]	9	[5.4%]	26**	[15.5%]	41**	[36%]
14th rib									
- unilateral	4–16	16	[10%]	24	[14.5%]	28	[16.7%]	18	[15.8%]
- bilateral	4–12	19	[11.9%]	17	[10.2%]	63**	[37.5%]	81**	[71.1%]
- unilateral/bilateral	8–27	35	[21.9%]	41	[24.7%]	91**	[54.2%]	99**	[86.8%]
Kinked ribs	—	—	—	—	—	—	—	2	[1.8%]
1st sternebra partially ossified	—	3	[1.9%]	3	[1.8%]	4	[2.4%]	12**	[10.5%]
2nd sternebra partially ossified	—	7	[4.4%]	11	[6.6%]	10	[6.0%]	24**	[21.5%]
2nd sternebra misaligned	—	—	—	1	[0.6%]	1	[0.6%]	—	—
Calcaneum not ossified									
- unilateral	3–5	10	[6.3%]	7	[4.2%]	3	[1.8%]	—	—
- bilateral	79–85	112	[70%]	137**	[82.5%]	157**	[93.5%]	114**	[100%]
- unilateral/bilateral	83–99	122	[76.3%]	144*	[86.7%]	160**	[95.2%]	114**	[100%]
Mean manus score[b]	1.9–2.59	2.42		2.66*		2.65*		3.13**	
Mean pes score[b]	2.4–3.05	2.72		3.06**		3.21**		3.63**	

From Killick et al. (1982)

* $P < 0.05$; ** $P < 0.01$ (Fisher's test)

[a] From five contemporary studies using the same diet (Moxon, 2005a).

[b] Based on a laboratory scale of 1–4 for ossification (1 = good; 4 = poor).

A subsequent developmental range-finding study was intended to investigate the embryo and fetal effects seen during the first study. Female Han Wistar rats (five per group) were administered flutriafol (purity 95.1%; batch No. UPL BX 1) by gavage at 0 (corn oil), 2, 5 or 100 mg/kg bw per day from gestation days 6 to 20. The dams were sacrificed on gestation day 21, and the fetuses were removed by caesarean section. External and skeletal examinations were performed for all fetuses in all dose groups.

At 100 mg/kg bw per day, there were significant reductions (~15%) in maternal body weight gain during the dosing period. There were also increases in post-implantation losses and fetal resorptions, with an associated reduction in litter size (9.2 versus 11.6 in controls). Fetuses from this group had increased incidences of skeletal anomalies, including absence of hyoid body, interrupted hyoid body, bent hyoid bodies, zygomatic arch fusions, pelvic girdle displacements, extra ribs and variations in the cervical ribs. One fetus had a cleft palate.

There were no treatment-related findings at 2 or 5 mg/kg bw per day. A higher rate of post-implantation loss at 5 mg/kg bw per day is not considered biologically relevant, as the mean number of live fetuses per litter was greater than in controls.

The maternal and fetal NOAEL for this limited study is 5 mg/kg bw per day. Cleft palate is a rare finding in rats, and its presence at low incidences in both of the preliminary developmental studies indicates, together with other fetal anomalies, that flutriafol is considered teratogenic at maternally toxic doses (Gerspach, 2008c).

Presumed pregnant female Han Wistar rats (22 per group) were orally administered flutriafol (purity 95.1%; batch No. UPL BX 1) by gavage at 0 (corn oil), 2, 5, 10 or 75 mg/kg bw per day from gestation days 6 to 20. Regular assessments were performed for clinical signs, body weight and feed consumption. The dams were sacrificed on gestation day 21, and the fetuses were removed by caesarean section and examined externally. Approximately half of the fetuses were examined for visceral and 50% for skeletal (alizarin red and alcian blue staining) effects. Dams were subjected to a gross necropsy examination. An expert peer review was performed on the hyoid findings.

Achieved doses were confirmed by analysis as satisfactory. No mortality or clinical signs of toxicity were noted in the dams. Test item–related effects were apparent only at 75 mg/kg bw per day. There was a reduction in initial body weight gain and in net body weight gain (minus uterine contents) (Table 24). Post-implantation loss was increased in the top-dose group (Table 24). At external examination, one control fetus had micrognathia and one top-dose fetus had a cleft palate. There were two treatment-related effects evident at visceral examination of top-dose animals: misshapen nasopharynx (three fetuses in three litters) and displaced origin of the common carotid artery (four fetuses in four litters). Increased incidences of several abnormalities and variations in the skeleton (particularly the hyoid) occurred at the top dose level (Table 24).

No treatment-related skeletal findings were observed at 2, 5 or 10 mg/kg bw per day. The high incidence of rudimentary supernumerary ribs at 5 and 10 mg/kg bw per day is within the contemporary historical control range (23–44% of fetuses). Sporadic increases in skeletal findings such as zygomatic arch fusion and pelvic girdle displacement exhibited no dose–response relationship and are not considered to be treatment related (Table 24).

The pathology peer review changed some of the descriptions of the findings but did not alter the conclusion that at 75 mg/kg bw per day, flutriafol produces a high incidence of skeletal abnormalities, particularly of the hyoid. The presence of cleft palate, together with other anomalies (e.g. of the hyoid), indicates that flutriafol is considered teratogenic at maternally toxic doses.

The maternal and developmental NOAEL is 10 mg/kg bw per day, based on reduced body weight gain and extensive skeletal effects at 75 mg/kg bw per day (Gerspach, 2008d; T. Kvieholm, personal communication, 2011).

Table 24. Findings in pregnant rats dosed with flutriafol

Finding	Fetus or litter	Dose (mg/kg bw per day)				
		0	2	5	10	75
Body weight gain, days 6–8 (g)		5	5	5	5	3**
Body weight gain, days 6–16 (g)		22	21	21	22	18**
Net weight gain, days 0–21 (g)		26	28	27	27	18**
Mean litter size		13.1	11.6	12.1	13.5	11.7
Post-implantation loss (%)		4.3	3.6	4.7	1.7	12.8**
Fetal resorptions (%)		0.3	0.8	0	0	7.1**
External examination (fetuses)		*288*	*244*	*266*	*296*	*258*
Cleft palate		0	0	0	0	1
Micrognathia		1	0	0	0	0
Skeletal examination (fetuses)		*139*	*117*	*129*	*142*	*123*
Hyoid body interrupted	Fetus	0	0	0	0	9**
	Litter	0	0	0	0	4*
Hyoid body bent	Fetus	0	0	0	0	2
	Litter	0	0	0	0	2
Hyoid body accentuated curvature	Fetus	0	0	0	0	42**
	Litter	0	0	0	0	16**
Squamosal or zygomatic process of maxilla, additional ossification	Fetus	0	0	0	0	10**
	Litter	0	0	0	0	7**
Zygomatic arch fusion	Fetus	4	0	7	1	61**
	Litter	3	0	6	1	22**
Maxilla or mandible blue-stained foci	Fetus	0	0	0	0	59**
	Litter	0	0	0	0	18**
Cervical rib long	Fetus	0	0	0	0	9*
	Litter	0	0	0	0	4
Cervical rib rudimentary	Fetus	0	2	2	1	36**
	Litter	0	2	2	1	17**
Pelvic girdle caudal displacement	Fetus	2	1	12	3	16*
	Litter	2	1	5	1	11
Incompletely ossified sternebra 6	Fetus	0	2	0	2	14*
	Litter	0	2	0	2	8*
Supernumerary ribs, left	Fetus	1	1	5	3	15**
	Litter	1	1	2	1	10**
Supernumerary rudimentary ribs, left	Fetus	34	35	48**	50**	94**
	Litter	12	14	19**	17	22**
Supernumerary ribs, right	Fetus	2	1	4	3	13**
	Litter	2	1	3	2	9**
Supernumerary rudimentary ribs, right	Fetus	31	30	41	47**	96**
	Litter	14	15	18	17	22**
Supernumerary rudimentary ribs, total	Fetus (%)	34 (24)	35 (30)	48 (37)**	50 (35)**	96 (78)**
	Litter	16	16	20	18	22*
Non-ossified proximal phalanges of toes						
- toe 2 proximal phalanx, left	Fetus	4	3	6	0	23**
	Litter	3	2	5	0	13**

Table 24 (continued)

Finding	Fetus or litter	Dose (mg/kg bw per day)				
		0	2	5	10	75
- toe 3 proximal phalanx, left	Fetus	4	1	4	0	22**
	Litter	3	1	3	0	12**
- toe 4 proximal phalanx, left	Fetus	4	2	4	0	21**
	Litter	3	1	3	0	11**
- toe 2 proximal phalanx, right	Fetus	4	3	5	0	23**
	Litter	3	2	4	0	13**
- toe 3 proximal phalanx, right	Fetus	4	2	4	0	23**
	Litter	3	1	3	0	12**
- toe 4 proximal phalanx, right	Fetus	4	2	4	0	21**
	Litter	3	1	3	0	11**

From Gerspach (2008d); T. Kvieholm, personal communication, 2011
* $P < 0.05$; ** $P < 0.005$

Rabbits

Mated female Dutch White rabbits were dosed orally (by capsule) with flutriafol (purity 93%; batch No. P10) at a dose level of 0, 2.5, 7.5 or 15 mg/kg bw per day on days 6–18 of gestation. Dams were observed daily for clinical signs; body weights were measured daily throughout the dosing period and at termination. Animals were sacrificed on day 29, and the uterine contents were investigated. All fetuses were examined for external findings, visceral malformations by dissection and skeletal findings following alizarin red staining.

Two top-dose dams were sacrificed during the study, one as a result of the presence of a large scab on the chin and thorax and one owing to "considerable" weight loss following the refusal of food and water. Gross necropsy indicated that the deaths were not directly attributable to treatment. One 7.5 mg/kg bw per day dam was sacrificed after aborting part of its litter. Treatment-related clinical signs were limited to a slightly increased incidence of loose faeces at the top dose level. Weight gains at the top dose level were lower than those of controls, significantly during the treatment phase and the post-treatment phase. No significant effects were seen on body weights. Feed consumption at the top dose level was also significantly lower during the treatment and post-treatment phases.

Mean corpora lutea and implantation numbers were comparable in all groups. Post-implantation loss (both early and late embryonic deaths) was significantly increased at the top dose level only, resulting in a significantly lower number of viable fetuses in this group. Findings are attributable to the significantly increased incidence of total resorptions (seen in five dams) in this group. Gravid uterus weight and litter weights were unaffected by treatment; mean fetal weight was slightly (but not significantly) lower at the top dose level (Table 25).

The total incidences of major external/visceral and skeletal fetal defects were slightly higher at 7.5 and 15 mg/kg bw per day; these findings are due to single fetuses with multiple findings. At the top dose level, the multiple malformed fetus is present in a litter with a significant level of resorptions, and together these are considered to be evidence of fetotoxicity and possible teratogenicity. A number of minor external/visceral and skeletal findings were also noted in these pups. Slightly increased incidences of minor skeletal parameters indicative of reduced ossification were noted at the top dose level, although values did not attain statistical significance. The incidence of extra (bilateral normal/short) lumbar ribs was also higher in this group; however, the total incidence of extra lumbar ribs was comparable in all treated groups and within the normal range for this strain of rabbit (13–50%; Moxon, 2005b). Mean scores for manus and pes ossification were slightly (but not significantly) higher at the top dose level, indicating marginally delayed or reduced ossification (Table 26).

Table 25. Maternal findings and litter parameters in rabbits administered flutriafol

Parameter / time point	Dose (mg/kg bw per day)			
	0	2.5	7.5	15
Maternal findings				
Body weight (g) [% of control]				
- day 0	2109	2143 [102%]	2172 [103%]	2145 [102%]
- day 6	2133	2207 [103%]	2220 [104%]	2221 [104%]
- day 19	2181	2240 [103%]	2254 [103%]	2142 [98%]
- day 29	2339	2427 [104%]	2416 [103%]	2294 [98%]
Weight gain (g) [% of control]				
- days 0–6	29.1	31.1 [107%]	30.8 [106%]	31.7 [109%]
- days 6–9[a]	6.4	10.4 [163%]	6.0 [93.8%]	3.0 [46.9%]
- days 9–12[a]	14.0	14.4 [101.4%]	17.0 [121.4%]	13.3 [95.0%]
- days 12–15[a]	21.2	19.0 [89.6%[	18.4 [86.8%]	14.5 [68.4%]
- days 6–15	41.6	43.6 [105%]	41.4 [100%]	30.8* [74.0%]
- days 15–21	89.2	86.1 [96.5%]	85.4 [95.7%]	60.2* [67.5%]
- days 6–21[a]	130.8	129.7 [99.2%]	126.8 [96.9%]	91.0 [69.6%]
- days 0–21[a]	159.9	160.8 [101%]	157.6 [98.6%]	122.7 [76.7%]
Feed consumption (g) [% of control]				
- days 0–6	24.2	24.5 [101%]	24.6 [102%]	24.8 [102%]
- days 6–15	22.6	22.4 [99.1%]	22.2 [98.2%]	18.8** [83.2%]
- days 15–21	35.5	35.0 [98.6%]	35.4 [99.7%]	30.6** [86.2%]
Mated (no.)	18	18	18	19
Not pregnant (no.)	2	2	1	3
Deaths (no.)	1	0	1	2
Aborted (no.)	0	0	1	0
Total resorptions (no.)	0	1	0	5*
Litters with viable fetuses (no.)	15	15	15	9
Litter parameters				
Corpora lutea (no.)	9.0	8.5	7.9	9.9
Implantations (no.)	7.1	7.0	6.4	8.1
Pre-implantation loss (%)	20.9	13.4	17.5	8.7
- litters affected	11/15	12/15	8/15	5/11
Post-implantation loss (%)	13.1	13.8	5.8	45.5*
- litters affected	6/15	8/16	4/15	8/14
- excluding total resorptions	13.1	8.0	5.8	15.3
Early embryonic death (%)	10.4	7.1	5.0	31.0
- litters affected	5/15	6/16	4/15	6/14
Late embryonic death (%)	1.0	2.7	1.0	16.4
- litters affected	1/15	2/16	1/15	3/14
Viable fetuses (no.)	6.5	6.3	6.0	4.0*
- excluding total resorptions	6.3	6.5	6.0	6.7
Gravid uterus weight (g)	285	316	286	307

Table 25 (continued)

Parameter / time point	Dose (mg/kg bw per day)			
	0	2.5	7.5	15
Litter weight (g)	208	224	208	223
Fetal weight (g)	34.0	34.8	35.6	32.0

From Kilmartin & Godley (1982)
* $P < 0.05$; ** $P < 0.01$ (*t*-test)
[a] Statistical analysis not performed.

Table 26. Fetal findings and litter parameters in rabbits administered flutriafol

Parameter	Dose (mg/kg bw per day)			
	0	2.5	7.5	15
Fetal incidence (%)				
Minor external and visceral defects	5 (5.3%)	6 (5.9%)	4 (4.3%)	4 (6.6%)
Major external and visceral defects	0	0	1 (1.1%)[a]	1 (1.6%)[b]
Minor skeletal defects	27 (28.7%)	23 (22.5%)	30 (31.9%)	26 (42.6%)
Major skeletal defects	0	0	1 (1.1%)[a]	1 (1.6%)[b]
Skeletal findings (%)				
Frontals: partially ossified	2 (2.1%)	2 (2.0%)	6 (6.4%)	8 (13.1%)
Parietals: partially ossified	6 (6.4%)	4 (3.9%)	3 (3.2%)	4 (6.5%)
Interparietals				
- partially ossified	1 (1.1%)	2 (2.0%)	0	4 (6.5%)
- not ossified	0	0	0	2 (3.3%)
Occipitals: partially ossified	2 (2.1%)	0	0	2 (3.3%)
7th lumbar vertebra: not ossified	0	0	0	1 (1.6%)
Extra lumbar ribs				
- unilateral short	4 (4.2%)	9 (8.8%)	4 (4.2%)	6 (9.8%)
- unilateral normal	3 (3.2%)	4 (3.9%)	1 (1.1%)	1 (1.6%)
- bilateral short	1 (1.1%)	4 (3.9%)	4 (4.2%)	3 (4.9%)
- bilateral normal	3 (3.2%)	10 (9.8%)	14 (14.9%)	9 (14.7%)
- bilateral normal/short	2 (2.1%)	2 (2.0%)	4 (4.2%)	7 (11.5%)
- total	13 (13.8%)	29 (28.4%)	27 (28.7%)	26 (42.6%)
Mean manus score	1.88	1.88	1.77	1.95
Mean pes score	1.88	1.91	1.75	1.95

From Kilmartin & Godley (1982)
[a] Single fetus with encephalocoele, shortened jaw, cleft palate, microphthalmia, gastroschisis, clubbed foot and malformed skull; the remainder were normal.
[b] Single fetus with encephalocoele, shortened jaw, gastroschisis, clubbed foot and malformed skull; the remainder were totally resorbed.

A maternal NOAEL of 7.5 mg/kg bw per day can be determined, based on the clinical signs and body weight effects at the top dose level. A developmental NOAEL of 7.5 mg/kg bw per day can also be determined, based on the significantly increased post-implantation loss, significantly lower number of viable fetuses and slightly increased incidences of a number of fetal skeletal parameters indicative of reduced or retarded ossification at 15 mg/kg bw per day (Kilmartin & Godley, 1982).

2.6 Special studies

(a) Acute neurotoxicity

Crl:CD(SD) rats (10 of each sex per group) received flutriafol (purity 95.1%, batch No. BX-1) at a single oral gavage dose of 0, 125, 250 or 750 mg/kg bw using a corn oil vehicle. Dose levels were based on a range-finding study, which reported adverse clinical signs in the 1st hour after dosing at 500 mg/kg bw and above, with reduced body weight gain evident at 125 mg/kg bw and above. Regular, routine observations included clinical signs and appearance. An extensive FOB was performed pre-test and on days 1 (8 hours), 8 and 15; motor activity was monitored over a 90-minute session. At sacrifice on day 16, animals were perfused with 10% formalin. Brain and spinal cord were removed. Peripheral nerve samples were prepared from five rats of each sex per group. A wide range of brain, spinal cord, peripheral nerve and skeletal muscle sections were prepared and stained using haematoxylin and eosin (H&E), toluidine blue, luxol fast blue, crystal violet or Bielschovski's silver stain, singly or in combination.

Achieved doses were confirmed by analysis. At 750 mg/kg bw, four males and two females showed severe signs of toxicity and were sacrificed on day 2 or 3. There were no reports of clinical signs at 250 mg/kg bw. Body weight, particularly in males, was reduced over the first few days post-dosing at all dose levels, possibly secondary to reduced feed consumption (Table 27). At day 14, body weights were similar in all groups other than 750 mg/kg bw males. FOB and motor activity findings on day 1 were limited to the 750 mg/kg bw group, which had ataxia, ptosis, gait abnormalities and reductions in amount of movement and time spent moving. At days 7 and 14, there were alterations in urination pattern and slow breathing in males at 250 and 750 mg/kg bw; these findings are equivocal, as they were not evident on day 1 or in the repeated-dose neurotoxicity study (see below) at a dose of 172 mg/kg bw per day, but showed consistency and are considered potentially relevant to treatment with flutriafol. Variations in grip strength were within normal ranges. There were no significant findings at necropsy. A reduction in absolute brain weight at 750 mg/kg bw was considered not to be of biological relevance, as relative brain weights were unaltered. There were no indications of overt neurotoxicity or damage to the central or peripheral nervous system.

The NOAEL for neuropathy was 750 mg/kg bw, the highest dose tested. The NOAEL for neurotoxicity was 250 mg/kg bw, based on altered FOB and motor activity at 750 mg/kg bw. A NOAEL for general toxicity was not determined, as transient reductions in body weight and feed consumption were seen in all dose groups at the start of the study (Barnett, 2006a).

(b) Repeated-dose neurotoxicity

In a range-finding repeated-dose neurotoxicity study, Crl:CD(SD) rats (five of each sex per dose group) received flutriafol (purity 95.1%, batch No. BX-1) in the diet at a concentration of 0, 1000, 2000 or 4000 ppm ad libitum for 29 days. On day 29, a 1-minute open-field examination was performed. At 4000 ppm (~85 mg/kg bw per day), there was initial body weight loss and reduced feed consumption. At 2000 ppm (~170 mg/kg bw per day), there was a transient reduction in feed consumption. There were no treatment-related clinical signs or effects on the results of the open-field examination (Barnett, 2006b).

In the main study, Crl:CD(SD) rats (10 of each sex per dose group) received flutriafol (purity 95.1%, batch No. BX-1) in the diet at a concentration of 0, 500, 1500 or 3000 ppm ad libitum for 13 weeks. FOB and motor activity (1.5-hour session split into 5-minute segments) evaluations were conducted on all rats before the 1st day of exposure and during weeks 2, 4, 8 and 13 of the exposure period. At sacrifice, animals were perfused with 10% formalin. Brain and spinal cord were removed. Peripheral nerve samples were prepared from five rats of each sex per group. A range of brain (six regions), spinal cord (three levels), peripheral nerve and skeletal muscle sections were prepared and

Table 27. Body weight and feed consumption in rats receiving flutriafol

	Dose (mg/kg bw)							
	0		125		250		750	
	Males	Females	Males	Females	Males	Females	Males	Females
Body weight (g)								
- day 1	252	186	253	187	254	182	254	187
- day 2	256	187	248	187	242	177	225*	177
- day 3	266	194	261	196	257	190	221*	181
- day 7	295	210	292	210	291	204	257*	208
Body weight gain (g)								
- days 1–2	3.9	1.5	−5.0*	−0.1	−12*	−4.2*	−29*	−10*
Feed consumption (g/day)								
- days 1–2	19	12	11*	9	7*	7*	2*	5*
- days 1–8	25	19	24	19	24	18	15*	17

From Barnett (2006a)
* $P < 0.05$

stained using H&E, toluidine blue, luxol fast blue, crystal violet or Bielschovski's silver stain, singly or in combination.

Achieved doses and homogeneity were confirmed by analysis; mean doses were 0, 29, 84 and 172 mg/kg bw per day in males and 0, 33, 98 and 185 mg/kg bw per day in females. There were no deaths or clinical signs of toxicity or any abnormal findings at necropsy. Significant reductions in body weight gain and feed consumption were seen at 1500 and 3000 ppm, with transient body weight loss at 3000 ppm. FOB and motor activity results were similar across all groups. Slight reductions in hindlimb grip strength at 3000 ppm appeared to be related to the lower body weight. There were no indications of damage to the central or peripheral nervous system.

The NOAEL for neuropathy and neurotoxicity was 3000 ppm (equal to 172 mg/kg bw per day), the highest dose tested. The NOAEL for general toxicity was 500 ppm (equal to 29 mg/kg bw per day), based on reductions in body weight gain at the start of the study at 1500 ppm (equal to 84 mg/kg bw per day (Barnett, 2007b).

(c) Immunotoxicity

Female CD-1 mice (10 per group) received flutriafol (purity 95.1%; batch No. UPL BX1) in the diet for 28 days at 0, 50, 250, 500 or 1000 ppm. A positive control group received cyclophosphamide (50 mg/kg bw per day) intraperitoneally on days 25–28. Mice were observed routinely for clinical signs, feed consumption, body weight and mortality. Four days prior to sacrifice, mice received an intravenous injection (tail vein) of sheep red blood cells to investigate immunoglobulin M (IgM)–mediated response to antibody. At sacrifice, a blood sample was taken for haematological investigation; liver, spleen and thymus were removed from all animals and weighed. The spleen was examined for immunological response, and the livers from negative control and top-dose groups were examined histopathologically.

Achieved mean doses were 0, 9.8, 47, 94 and 208 mg/kg bw per day. There were no deaths, clinical signs or alterations in feed consumption. Transient body weight loss was seen at and above 500 ppm (Table 28). Absolute and relative liver weights were increased at and above 250 ppm, with periacinar hypertrophy and vacuolation present in all livers examined histopathologically (1000 ppm group only). Altered erythrocyte parameters were found at and above 250 ppm, progressing with

Table 28. Effects in female mice exposed to flutriafol in an immunotoxicity study

	Dietary concentration (ppm)					
	0	50	250	500	1000	CPA[a]
Body weight gain, days 1–4 (g)	0.6	0.4	0.5	−0.5	−1.7	—
Relative liver weight (%)	5.5	5.6	7.7*	10.8*	16.5*	5.4
Relative spleen weight (%)	0.46	0.45	0.41	0.46	0.49	0.24*
Relative thymus weight (%)	0.20	0.23	0.19	0.21	0.19	0.08*
Platelets (10^3/mm^3)	925	1250*	1130	1283*	1264*	—
White blood cells (10^3/mm^3)	6.8	7.3	8.9	11.0*	13.4*	—
IgM response (antibody-forming cells/10^6 spleen cells)	1626	1566	1309	1450	2000	0

From Barnett (2011)
CPA, cyclophosphamide; IgM, immunoglobulin M; * $P < 0.05$
[a] 50 mg/kg bw per day intraperitoneally.

administered dose. Increases in white blood cell counts were seen in all groups, primarily due to increases in lymphocyte and segmented neutrophils, achieving statistical significance at and above 500 ppm. Platelet counts were higher in all treated groups, but showed no dose–response relationship and were consistent with the control range ($407 \times 10^3 – 1491 \times 10^3$/mm^3). There were no effects on spleen or thymus weights or on the measured immunological response (Table 28). The positive control results confirmed the viability of the test system.

The NOAEL for general toxicity (within the limited investigations in the study) is 50 ppm (equal to 9.8 mg/kg bw per day), based on haematological changes and hepatotoxicity at 250 ppm (equal to 47 mg/kg bw per day). Flutriafol was not immunotoxic in this study, with a NOAEL of 1000 ppm (equal to 208 mg/kg bw per day) (Barnett, 2011).

3. Observations in humans

No reports of intentional or accidental poisoning with flutriafol have been found in the published literature. Health monitoring of production plant workers has not identified any cases of illness associated with flutriafol (Denman, 1994; McNie, 2002; I.M. Jensen, personal communication, 2011).

Comments

Biochemical aspects

Flutriafol is rapidly and extensively absorbed following oral administration to the rat at a dose level of 5 or 250 mg/kg bw in PEG600. Based on values for urinary and biliary excretion, oral absorption is greater than 90%. Flutriafol and/or its metabolites are widely distributed, with highest levels of radioactivity associated with red blood cells. Data from studies with repeated administration indicate that there is unlikely to be any bioaccumulation. Flutriafol is extensively metabolized in the rat, with only trace levels of unchanged parent detected in excreta following oral administration. The initial stage of metabolism is oxidation of the 2-fluorophenyl ring, followed by conjugation. Excretion was predominantly within 24 hours at 5 mg/kg bw, with similar amounts of radiolabel present in the urine and faeces. Biliary excretion was extensive (~80%), with evidence for enterohepatic circulation.

Toxicological data

Flutriafol has acute oral LD_{50} values of 1140–1480 mg/kg bw in the rat. Acute oral toxicity was higher in the mouse, rabbit and guinea-pig (LD_{50}s 179–400 mg/kg bw). Lower acute toxicity

was seen via the dermal (LD_{50} > 2000 mg/kg bw in rabbits) and inhalation (LC_{50} > 5.2 mg/l) routes. Flutriafol was not irritating to rat or rabbit skin but was a mild irritant to rabbit eyes; all ocular effects had resolved within 72 hours. Flutriafol did not produce any evidence of skin sensitization in a Magnusson and Kligman assay in guinea-pigs or in a local lymph node assay in mice.

The red blood cell and liver were identified as targets of flutriafol toxicity in short-term oral toxicity studies in the rat, mouse and dog. Increases in hepatocyte hypertrophy and liver weight with no other effects were considered to be adaptive and not treated as adverse effects; other histopathological findings in the liver, such as fatty vacuolation or necrosis, were treated as adverse. Reduced body weights, weight gains and/or feed consumption were also seen at high dose levels in all three species. Effects on haematological parameters consistent with mild microcytic anaemia were seen in the rat and mouse; such effects were less marked in the dog. In a 29-day study, the majority of mice exposed at 1500 ppm died. Increased liver weights and hepatocytic vacuolation were seen at intermediate doses. Hepatocytic lipid accumulation was seen at all dose levels; the lowest-observed-adverse-effect level (LOAEL) was 50 ppm (equivalent to 7.5 mg/kg bw per day). Hepatotoxicity in the rat at high dose levels was characterized by the disruption of lipid metabolism, increased liver weight, altered pigmentation and granulation, centrilobular hepatocyte hypertrophy and vacuolation, fatty change, focal necrosis and hydropic degeneration. At lower dose levels, findings of increased liver weight, centrilobular hepatocyte hypertrophy, elevated hepatic aminopyrine *N*-demethylase activity and proliferation of the smooth endoplasmic reticulum are consistent with an adaptive effect. The NOAEL in the 90-day rat study was 200 ppm (equal to 13 mg/kg bw per day). In the dog, hepatotoxicity was characterized by elevated serum alkaline phosphatase activity, perturbation of lipid metabolism, increased liver weight and (in the 1-year study) granular, swollen liver, hepatocytic vacuolation and lipid accumulation. Initial reductions in body weight and body weight gain with no associated reductions in feed consumption were seen at dose levels above 5 mg/kg bw per day. The NOAELs for the 90-day and 1-year dog studies were both 5 mg/kg bw per day.

In a 28-day dermal toxicity study in rats, there were no systemic effects; the NOAEL was 1000 mg/kg bw per day. The NOAEL for local effects was 250 mg/kg bw per day, based on erythema and flaking of the skin at the application site at 500 mg/kg bw per day.

The liver was identified as the target organ of flutriafol toxicity following chronic administration to the rat and mouse. Hepatotoxicity in the 2-year mouse study was characterized by increased liver weight and fatty change; the incidence of liver adenoma was marginally increased in males at the top dose level, but was within the historical control range. The NOAEL in the 2-year mouse study was 10 ppm (equal to 1.2 mg/kg bw per day), based on centrilobular fatty changes in the liver at 50 ppm (equal to 6 mg/kg bw per day). Flutriafol was not carcinogenic in mice.

Toxicity in the chronic rat study was characterized by clinical chemistry findings (reduced serum triglycerides and ALP activity and increased ALT activity in males; increased serum cholesterol in females) at 2000 ppm and increased liver weight and fatty change at 200 ppm and above. Incidences of liver adenoma and carcinoma were slightly increased at the top dose level, but were within historical control ranges. Evidence of anaemia was seen at the high dose level; effects on red blood cell parameters were accompanied in females by reduced serum iron concentration, elevated total iron binding capacity and haemosiderin accumulation in the spleen and liver. The NOAEL in the 2-year rat study was 20 ppm (equal to 1.0 mg/kg bw per day), based on increased fatty change and weight of the liver in males at 200 ppm (equal to 10 mg/kg bw per day). Flutriafol was not carcinogenic in rats.

The Meeting concluded that flutriafol is not carcinogenic in mice or rats.

Flutriafol has been tested for genotoxicity in an adequate range of in vitro and in vivo studies. Equivocal results were seen in two reverse mutation assays with mouse lymphoma L5178Y cells. The remaining in vitro and all the in vivo tests were negative.

The Meeting concluded that flutriafol is unlikely to be genotoxic.

Based on the absence of genotoxicity and absence of treatment-related carcinogenicity in mice and rats, the Meeting concluded that flutriafol is unlikely to be carcinogenic in humans.

Two reproductive toxicity studies in rats are available. Evidence of reproductive toxicity was seen in the first two-generation rat study; mean litter size was lower at the top dose level of 1000 ppm, and there was a reduced fertility index at the mating for the F_{1A} litter. The NOAEL for reproductive toxicity was 240 ppm (equal to 14 mg/kg bw per day). Minor maternal body weight effects and hepatotoxicity (fatty change) were seen at 240 ppm in both sexes. The NOAEL for parental toxicity was 60 ppm (equal to 3.5 mg/kg bw per day). Fatty changes in the liver were also seen in pups at the top dose level. The NOAEL for offspring toxicity was 240 ppm (equal to 14 mg/kg bw per day). Similar findings were seen in the range-finding component of the more recent rat reproductive toxicity study. Post-implantation losses (13%) were increased at doses causing mild to moderate maternal toxicity in the preliminary reproduction study at 240 ppm (equal to 13 mg/kg bw per day) and above. NOAELs for parental, pup and reproductive effects were 60 ppm (approximately 4 mg/kg bw per day). However, in the main study, there were no effects on reproduction, offspring or parents at the highest dose tested, 300 ppm (equal to 16 mg/kg bw per day). The Meeting concluded that the overall NOAELs from the two main reproductive toxicity studies for reproductive and offspring toxicity were 16 mg/kg bw per day.

There are four rat developmental toxicity studies on flutriafol. In the first guideline-compliant study, effects on the fetal skeleton mainly consistent with delayed ossification were seen at all dose levels; the LOAEL was 10 mg/kg bw per day. At the top dose level of 125 mg/kg bw per day, there were reductions in body weight gain (20–50%), feed consumption (~15%) and litter size (30%), the latter associated with increased post-implantation loss (33%). In both subsequent range-finding studies, there were marked increases in skeletal anomalies (especially of the hyoid) and reduced ossification, as well as single incidences of cleft palate at the top dose levels of each study (150 and 100 mg/kg bw per day). Marked maternal toxicity, clinical signs, body weight deficits and fetal resorptions were seen at the top dose levels. In the second guideline-compliant study, there was a single incidence of cleft palate at the top dose level of 75 mg/kg bw per day, a dose that also produced significantly reduced maternal body weight gain (~30%), increases in post-implantation loss (3-fold), skeletal anomalies (including the hyoid bone and supernumerary ribs) and delayed ossification. The NOAELs in this study for both developmental and maternal toxicity were 10 mg/kg bw per day.

Cleft palate is a very rare finding in rats that has been seen at high dose levels with a number of triazole compounds. The presence of single incidences of cleft palate in three of the rat developmental toxicity studies, although not statistically significant in isolation, cannot be discounted when the database is considered as a whole. Litter sizes at the top dose levels were significantly lower as a result of post-implantation loss, which could reduce the number of malformed fetuses observed at caesarean section. The NOAEL for teratogenicity in the rat was 10 mg/kg bw per day, based on the findings of cleft palate at and above 75 mg/kg bw per day.

In a rabbit developmental toxicity study, there was evidence of clinical signs, reduced maternal body weight gain (30%), reduced litter size (40%) and delayed ossification at the highest dose level of 15 mg/kg bw per day. One fetus at the top dose level and one at the intermediate dose level had multiple malformations that could not be unequivocally linked to flutriafol administration. The NOAELs for maternal and developmental toxicity were 7.5 mg/kg bw per day.

The Meeting concluded that flutriafol is teratogenic in rats.

In an acute neurotoxicity study in rats, there was no evidence of neuropathy at 750 mg/kg bw, the highest dose tested. The NOAEL for acute neurotoxicity was 250 mg/kg bw, based on altered FOB and motor activity findings on day 1 at 750 mg/kg bw. The NOAEL for general toxicity was less than 125 mg/kg bw, based on transient reductions in body weight gain in males at all doses.

In a repeated-dose neurotoxicity study, there were no signs of neuropathy or neurotoxicity at 3000 ppm (equal to 172 mg/kg bw per day), the highest dose tested. The NOAEL for general toxicity

was 500 ppm (equal to 29 mg/kg bw per day), based on reductions in body weight gain at the start of the study at 1500 ppm (equal to 84 mg/kg bw per day) and above.

In a 28-day immunotoxicity study in female mice, there was no reduction in the IgM response to challenge with sheep red blood cells or changes in spleen or thymus weights. The NOAEL for immunotoxicity was 1000 ppm (equal to 208 mg/kg bw per day), the highest dose tested. The NOAEL for general toxicity was 50 ppm (equal to 9.8 mg/kg bw per day), based on reduced erythrocyte mean cell volume and hepatotoxicity at 250 ppm (equal to 47 mg/kg bw per day).

Medical monitoring of production plant workers has not identified any cases of occupational illness related to flutriafol.

The Meeting concluded that the available database on flutriafol is adequate to characterize the potential risk to fetuses, infants and children.

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) of 0–0.01 mg/kg bw on the basis of the NOAEL of 1.0 mg/kg bw per day in the 2-year rat study, based on increases in fatty changes and increased weights of the liver in males at 10 mg/kg bw per day. A safety factor of 100 was applied. The ADI is supported by the NOAEL in the carcinogenicity study in mice of 1.2 mg/kg bw per day, based on the increased incidence and severity of hepatic centrilobular fatty change in males at 6 mg/kg bw per day.

The Meeting established an acute reference dose (ARfD) of 0.05 mg/kg bw on the basis of the NOAEL of 5 mg/kg bw per day in the 90-day and 1-year toxicity studies in dogs based on reduced body weight gain (males) or body weight loss (females) after 1 week (the first time of measurement) and subsequently reduced body weight gain during the early part of the study, although feed consumption was unaffected by treatment. A safety factor of 100 was applied. This provides a margin of greater than 1000 between the ARfD and the LOAEL for cleft palate in rats (75 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	10 ppm, equal to 1.2 mg/kg bw per day	50 ppm, equal to 6 mg/kg bw per day
		Carcinogenicity	200 ppm, equal to 25 mg/kg bw per day[b]	—
Rat	Ninety-day study of toxicity[a]	Toxicity	200 ppm, equal to 13 mg/kg bw per day	2000 ppm, equal to 148 mg/kg bw per day
	Two-year study of toxicity and carcinogenicity[a]	Toxicity	20 ppm, equal to 1.0 mg/kg bw per day	200 ppm, equal to 10 mg/kg bw per day
		Carcinogenicity	2000 ppm, equal to 103 mg/kg bw per day[b]	—
	Multigeneration studies of reproductive toxicity[a,c]	Reproductive toxicity	300 ppm, equal to 16 mg/kg bw per day	1000 ppm, equal to 56 mg/kg bw per day
		Parental toxicity	60 ppm, equal to 3.5 mg/kg bw per day	240 ppm, equal to 14 mg/kg bw per day
		Offspring toxicity	300 ppm, equal to 16 mg/kg bw per day	1000 ppm, equal to 56 mg/kg bw per day
	Developmental toxicity study[d]	Maternal toxicity	10 mg/kg bw per day	75 mg/kg bw per day
		Embryo and fetal toxicity	10 mg/kg bw per day	75 mg/kg bw per day

Species	Study	Effect	NOAEL	LOAEL
Rabbit	Developmental toxicity study[d]	Maternal toxicity	7.5 mg/kg bw per day	15 mg/kg bw per day
		Embryo and fetal toxicity	7.5 mg/kg bw per day	15 mg/kg bw per day
Dog	Ninety-day and 1-year studies of toxicity[c,e]	Toxicity	5 mg/kg bw per day	15 mg/kg bw per day

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

Estimate of acceptable daily intake for humans

0–0.01 mg/kg bw

Estimate of acute reference dose

0.05 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 flutriafol

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid and extensive absorption: > 90% based on urinary and biliary excretion
Dermal absorption, rat (125 g/l suspension concentrate formulation)	~1% concentrate; ~10% in use dilutions; 8 h exposure and 9-day monitoring
Distribution	Widely distributed; highest levels in red blood cells
Potential for accumulation	No evidence for accumulation
Rate and extent of excretion	Rapid, equally in urine and faeces; extensive biliary excretion (~80%) with enterohepatic circulation
Metabolism in animals	Extensive metabolism; only trace amount of unchanged parent detected
Toxicologically significant compounds (animals, plants and the environment)	Flutriafol

Acute toxicity	
Rat, LD_{50}, oral	1140–1480 mg/kg bw
Rabbit, LD_{50}, dermal	> 2000 mg/kg bw
Rat, LC_{50}, inhalation	> 5.2 mg/l
Rabbit, dermal irritation	Non-irritant
Rabbit, ocular irritation	Mild irritant
Guinea-pig, dermal sensitization (Magnusson and Kligman)	No evidence

Mouse, dermal sensitization (local lymph node assay)	No evidence

Short-term studies of toxicity

Target/critical effect	Body weights (from start of dosing); red blood cells (anaemia) and liver (weight, clinical chemistry, fatty change)
Lowest relevant oral NOAEL	5 mg/kg bw per day (90-day and 1-year dog studies)
Lowest relevant dermal NOAEL	1000 mg/kg bw per day (systemic; rats)
Lowest relevant inhalation NOAEC	No data

Long-term studies of toxicity and carcinogenicity

Target/critical effect	Liver (increased weight and fatty change)
Lowest relevant NOAEL	1.0 mg/kg bw per day (rat); 1.2 mg/kg bw per day (mouse)
Carcinogenicity	Not carcinogenic

Genotoxicity

	Some equivocal in vitro results; negative in vivo; considered unlikely to be genotoxic

Reproductive toxicity

Reproduction target/critical effect	Reduced litter size
Lowest relevant reproductive NOAEL	16 mg/kg bw per day
Developmental target/critical effect	Reduced litter size, delayed ossification (rat and rabbit); hyoid abnormalities (rat); cleft palate (rat)
Lowest relevant developmental NOAEL	7.5 mg/kg bw per day (rabbit); 10 mg/kg bw per day (rat)

Neurotoxicity/delayed neurotoxicity

Acute study	Altered FOB and motor activity; neurotoxicity NOAEL 250 mg/kg bw (rat)
Ninety-day study	Not neurotoxic

Other toxicological studies

Immunotoxicity	Not immunotoxic (female mice)

Medical data

	No adverse effects reported in production plant operators

Summary

	Value	Study	Safety factor
ADI	0–0.01 mg/kg bw	Two-year rat study (supported by 2-year mouse study)	100
ARfD	0.05 mg/kg bw	Ninety-day and 1-year studies in dogs	100

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GLYPHOSATE (addendum)

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Explanation

Glyphosate (*N*-(phosphonomethyl)glycine) is a non-selective systemic herbicide that was last evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 2004, when a group acceptable daily intake (ADI) for glyphosate and aminomethylphosphonic acid (AMPA), the main metabolite of glyphosate, of 0–1 mg/kg body weight (bw) was established based on a no-observed-adverse-effect level (NOAEL) of 100 mg/kg bw per day for salivary gland alterations in a long-term study of toxicity and carcinogenicity in rats and a safety factor of 100. The 2004 JMPR concluded that it was not necessary to establish an acute reference dose (ARfD) for glyphosate.

Metabolism studies in genetically modified soya beans and maize containing the glyphosate-*N*-acetyltransferase (*GAT*) gene demonstrated that new metabolites are formed that were not observed in conventional crops. The major metabolite in the new maize and soya bean varieties was *N*-acetyl-glyphosate (which may be degraded to glyphosate in the rat), whereas glyphosate, *N*-acetyl-AMPA and AMPA were found in low concentrations in the edible parts of the crops. The present Meeting was asked by the Codex Committee on Pesticide Residues to evaluate newly submitted studies on toxicokinetics and metabolism, acute oral toxicity, subchronic toxicity and genotoxicity for *N*-acetyl-glyphosate and on acute oral toxicity and genotoxicity for *N*-acetyl-AMPA.

All pivotal studies were certified as complying with good laboratory practice or an approved quality assurance programme.

Evaluation for acceptable daily intake

1. *N*-Acetyl-glyphosate (metabolite of glyphosate in genetically modified plants)

In a study on toxicokinetics and metabolism conducted according to United States Environmental Protection Agency test guideline OPPTS 870.7485, 45 male Sprague-Dawley rats (Crl:CD(SD)IGS BR) received [^{14}C]*N*-acetyl-glyphosate, sodium salt (purity 84.3%; radiochemical purity 99.2%), as a solution in water by oral gavage at a single dose of 15 mg free acid equivalent per kilogram body weight; the dose volume was 5 ml/kg bw. A correction factor of 0.674 was used to derive the free acid equivalent from the weight of the test substance. Blood was collected from four animals pre-dosing and from four animals per time point at 0.5, 1, 2, 4, 8, 12, 24, 48 and 72 hours post-dosing. Excreta were collected from five animals at specified intervals through 168 hours post-dosing. Plasma, excreta and carcass were analysed for content of radioactivity using liquid scintillation counting. Selected samples of plasma, urine and faeces were analysed for unchanged parent compound and metabolites.

The mean total recovery of radioactivity was 95.5%, with 66.1% in urine, 26.4% in faeces, 2.79% in cage wash and wipe and 0.23% in residual carcass (the values do not include data for one animal [C16498] that had suspected urine contamination of faeces). More than 90% of the total radioactivity was eliminated by 48 hours post-dosing.

The mean maximum concentrations (C_{max}) in blood and plasma were 2.93 and 5.31 µg equivalent (eq) per gram at 1 and 2 hours post-dosing, respectively. Radioactivity was eliminated from blood and plasma with half-life values of 20.1 and 15.6 hours, respectively. Comparison of blood and plasma values for area under the curve ($AUC_{0-\infty}$ = 12.1 and 20.8 µg eq·h/g, respectively) indicates that [^{14}C]*N*-acetyl-glyphosate distributed preferentially into plasma.

Unchanged [^{14}C]*N*-acetyl-glyphosate recovered in urine and faeces represented over 99% of the administered radioactivity. A metabolite, glyphosate, was detected in faeces and represented less than 1% of the total radioactivity. Plasma radioactivity consisted entirely of unchanged [^{14}C]*N*-acetyl-glyphosate (Cheng & Howard, 2004).

In an acute oral toxicity study conducted according to Organisation for Economic Co-operation and Development (OECD) Test Guideline 423 (Acute Toxic Class Method), five male and five female Sprague-Dawley rats (Crl:CD(SD)IGS BR) received *N*-acetyl-glyphosate, sodium salt (purity 84.3%), as a solution in water by oral gavage at a single dose of 5000 mg free acid equivalent per kilogram body weight (limit test); the dose volume was 10 ml/kg bw. A correction factor of 0.674 was used to derive the free acid equivalent from the weight of the test substance. Surviving animals were observed for clinical signs of toxicity, body weight effects and mortality for up to 14 days after dosing. All rats were examined for gross pathological changes.

Mortality was observed in one female at the 4-hour post-dosing observation and in one male and one female on the day after dosing. Clinical signs of toxicity observed in male and female rats included hypoactivity, irregular respiration, liquid or soft faeces, light-brown perineal staining, brown nasal crust and/or squinted eyes. All clinical signs of toxicity were resolved in all surviving animals by day 3 (2 days after dosing). All surviving animals gained weight from the initiation of dosing to study termination. At necropsy, findings were noted in the one male and two females that were found dead prior to the terminal sacrifice; however, no abnormal findings were noted in the remaining animals that survived to terminal sacrifice. Findings involved the lungs (mottled or discoloured bright red), liver (discoloured black), stomach (soft and/or with yellow fluid or gel-like clear liquid and red walls), abdominal cavity (clear fluid or reddish liquid) and duodenum, jejunum and ileum (fluid).

Under the conditions of this study, the oral median lethal dose (LD_{50}) of *N*-acetyl-glyphosate in rats was greater than 5000 mg/kg bw. The mortality rate was 20% in males and 40% in females dosed at 5000 mg/kg bw (Vegarra, 2004).

In a study of toxicity conducted according to OECD Test Guideline 408, groups of 10 male and 10 female Crl:CD(SD) rats were fed diets containing *N*-acetyl-glyphosate, sodium salt (purity 81.8%), at a concentration of 0, 180, 900, 4500 or 18 000 parts per million (ppm) (expressed as free acid equivalent), equal to 0, 11.3, 55.7, 283 and 1157 mg free acid equivalent per kilogram body weight per day in males and 0, 13.9, 67.8, 360 and 1461 mg free acid equivalent per kilogram body weight per day in females, for 90 days. A correction factor of 0.63 was used to derive the free acid equivalent from the weight of the test substance. Parameters evaluated included body weight, body weight gain, feed consumption, feed efficiency, clinical signs, ophthalmological evaluations, neurobehavioural evaluations (abbreviated functional observational battery [FOB], including forelimb and hindlimb grip strength, motor activity evaluation), haematology, clinical chemistry, urinalysis, organ weights, and gross and microscopic pathology. At test days 82 and 83 for male and female rats, respectively, blood and urine samples were collected and processed for the analyses of *N*-acetyl-glyphosate (IN-MCX20) and its metabolites, glyphosate (DPX-B2856) and *N*-acetyl-AMPA (IN-EY252). Urine samples were pooled prior to analysis, and individual blood samples were processed to produce plasma for subsequent analyses. Pooled urine and individual plasma samples were stored frozen until analysed. The analytes were quantified by high-performance liquid chromatography with detection by tandem mass spectrometry (LC-MS/MS).

No test substance–related mortality or clinical signs of toxicity were observed for any dietary concentration in either males or females. The death of one male rat at 4500 ppm on study day 42 was considered accidental, based on necropsy findings.

There were no adverse test substance–related effects on body weights or body weight gains in females, whereas in males at 18 000 ppm, body weights and body weight gains were decreased (92% and 86% of control, respectively) (Table 1). Feed efficiency was slightly reduced in males and females at 18 000 ppm (92% of control).

No test substance–related effects were observed on ophthalmology or neurobehavioural parameters (FOB, including forelimb and hindlimb grip strength, motor activity evaluation).

No test substance–related effects were observed on clinical pathology, organ weights, or gross or microscopic pathology.

The urinary concentrations of *N*-acetyl-glyphosate (IN-MCX20) increased with the increasing dietary levels of this test substance (Table 1). Concentrations of glyphosate (DPX-B2856) and *N*-acetyl-AMPA (IN-EY252) were detected above the limit of detection at higher dietary levels (900–18 000 ppm), but at or below the limit of detection in urine samples from the 180 ppm dietary group. Further, the concentrations of these metabolites were much higher in urine samples from male rats than from corresponding female rats at 4500 and 18 000 ppm. Neither *N*-acetyl-glyphosate nor either of its metabolites was detected in urine from control rats.

The plasma concentrations of *N*-acetyl-glyphosate also increased with the increasing dietary levels of this test substance (Table 1). Concentrations of *N*-acetyl-glyphosate were less than 1.0 µg/ml for males and females at 180 ppm and increased from a mean of approximately 2 µg/ml up to approximately 15 µg/ml for the other dietary groups. In contrast to urine samples, little to no glyphosate or *N*-acetyl-AMPA was detected in plasma for all dietary levels. Neither *N*-acetyl-glyphosate nor either of its metabolites was detected in plasma from control rats.

The proposed pathway for the metabolism of *N*-acetyl-glyphosate in rats is outlined in Figure 1.

In conclusion, the analytical results from urine and plasma samples demonstrated that *N*-acetyl-glyphosate was metabolized in rats to small quantities of glyphosate and *N*-acetyl-AMPA.

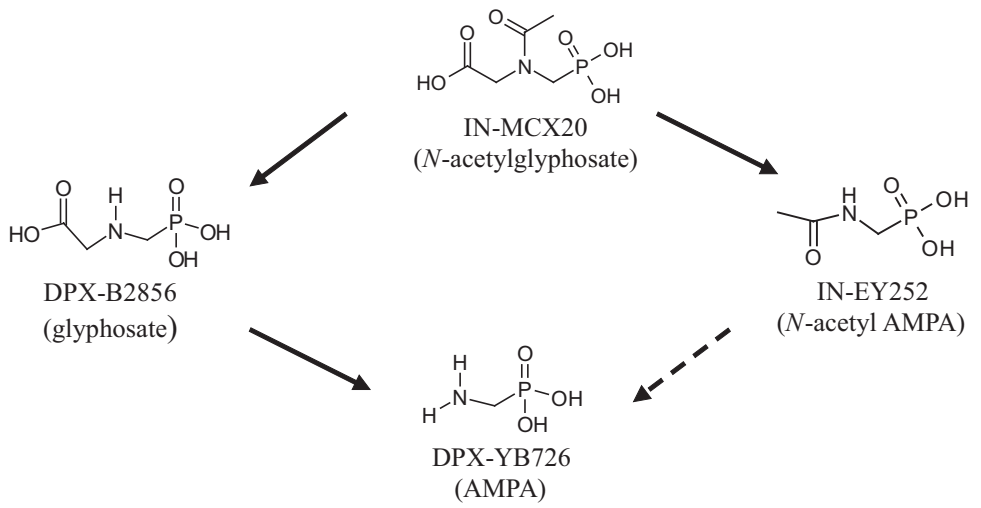
The NOAEL for the subchronic toxicity of *N*-acetyl-glyphosate was 4500 ppm (equal to 283 mg/kg bw per day), based on slightly decreased body weight gain in male rats at 18 000 ppm (equal to 1157 mg/kg bw per day) (MacKenzie, 2007; Shen, 2007).

Table 1. Summary of selected findings

	Males					Females				
	Dietary concentration (ppm)									
	0	180	900	4500	18 000	0	180	900	4500	18 000
Dose (mg/kg bw per day)	0	11.3	55.7	283	1157	0	13.9	67.8	360	1461
Body weight, day 91 (g)	583	563	575	559	534	287	303	294	303	283
Body weight gain, days 0–91 (g)	347	328	338	324	299*	111	127	121	127	107
Feed consumption, days 0–91 (g/animal per day)	29.8	28.3	28.3	28.2	27.8	19.4	20.2	19.4	21.2	20.3
Daily feed efficiency, days 0–91 (g body weight gain/g feed consumed)	0.128	0.127	0.131	0.126	0.118	0.063	0.069	0.068	0.066	0.058
Urine										
N-Acetyl-glyphosate (µg/ml)	ND	53.8	361	1150	2220	ND	71.5	360	1110	2020
Glyphosate (µg/ml)	ND	< 0.05	0.165	27.1	64.4	ND	< 0.05	0.360	2.92	4.02
N-Acetyl-AMPA (µg/ml)	ND	< 0.05	0.127	1.50	5.38	ND	< 0.05	0.179	< 0.5	2.89
Plasma										
N-Acetyl-glyphosate (µg/ml)	ND	0.33	1.85	4.77	14.83	ND	0.44	2.32	8.35	13.46
Glyphosate (µg/ml)	ND	ND	ND	ND	ND	ND	ND	ND	0.32	< 0.1
N-Acetyl-AMPA (µg/ml)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

From MacKenzie (2007); Shen (2007)
ND, not detected; * $P > 0.05$

Figure 1. Proposed metabolism of N-acetyl-glyphosate (IN-MCX20) in rats



Note: The metabolite AMPA (DPX-YB726) was not detected in the submitted rat studies with *N*-acetyl-glyphosate.
Source: Shen (2007)

In a reverse gene mutation assay in bacteria conducted according to OECD Test Guideline 471, *Salmonella typhimurium* (strains TA98, TA100, TA1535 and TA1537) and *Escherichia coli* (strain WP2*uvr*A) were exposed to *N*-acetyl-glyphosate, sodium salt (purity 84.3%), using deionized water as solvent, in the presence and absence of S9 metabolic activation. A correction factor of 0.674 was used to derive the free acid equivalent from the weight of the test substance, and all test substance

concentrations are expressed in terms of the free acid. Nominal concentrations of 100, 333, 1000, 3330 and 5000 µg/plate were evaluated in two trials using standard plate incorporation methods. The highest dose level was set based on the results of a dose range–finding study using tester strains TA100 and WP2*uvr*A and 10 doses of test substance ranging from 6.67 to 5000 µg/plate.

There was no evidence of cytotoxicity by the test compound. The number of revertants at all concentrations of the test substance was similar to that for concurrent controls in trials both with and without activation, with the exception of a 3.3-fold increase observed with tester stain TA1537 treated with 333 µg test substance per plate in the absence of S9 mix. However, this increase was not dose-responsive and did not meet the criteria for a positive evaluation. As the vehicle control value for tester strain TA98 in the absence of S9 mix was not in the acceptable range in one of the earlier trials, a third trial with TA98 was run in the absence of S9, with no increase in the number of revertants per plate. Under the conditions of this study, *N*-acetyl-glyphosate was considered to be negative for mutagenic activity in non-activated and S9-activated bacterial test systems (Mecchi, 2004).

In an in vitro mammalian cell gene mutation test conducted according to OECD Test Guideline 476, *N*-acetyl-glyphosate, sodium salt (purity 81.8%), dissolved in water was tested for its ability to induce forward mutations at the *HPRT* locus in Chinese hamster ovary (CHO) cells. A correction factor of 0.63 was used to derive the free acid equivalent from the weight of the test substance, and all test substance concentrations are expressed in terms of the free acid. Two independent sets of experiments were conducted in the presence and absence of S9 metabolic activation. Based on the results from a preliminary cytotoxicity assay, concentrations of 250–2091 µg/ml were used in the mutagenesis assay both with and without metabolic activation, and the same concentrations were used for the independent repeats. Ethyl methanesulfonate (EMS) and benzo(*a*)pyrene (BaP) served as positive controls in the experiments without and with metabolic activation, respectively. The cells were treated for 5 hours in both experiments, without and with metabolic activation. At the end of the exposure period, cells were washed and subcultured at 2- to 3-day intervals for a 7-day expression period. This was followed by incubation of the cells for 9 days in selection medium containing 6-thioguanine.

No visible precipitate in the treatment medium and no substantial toxicity were observed at any concentration in either non-activated or activated test systems. No increases in mutant frequencies were observed in any test substance treatment group, whereas the positive control substances EMS and BaP resulted in a marked increase in mutant frequency. Under the conditions of this study, *N*-acetyl-glyphosate was considered to be not mutagenic in the CHO/HPRT forward mutation assay, in either the presence or absence of metabolic activation (Glatt, 2006).

In an in vitro mammalian chromosomal aberration test conducted according to OECD Test Guideline 473, the clastogenic potential of *N*-acetyl-glyphosate, sodium salt (purity 84.3%), using deionized water as solvent, was tested in CHO cells in the presence and absence of S9 metabolic activation. A correction factor of 0.674 was used to derive the free acid equivalent from the weight of the test substance, and all test substance concentrations are expressed in terms of the free acid. Negative and vehicle controls and positive controls (cyclophosphamide and mitomycin C for the tests with and without metabolic activation, respectively) were included to demonstrate the sensitivity of the test system. The treatment period was for 3 hours with and without metabolic activation, and cultures were harvested approximately 20 hours from the initiation of treatment. In addition, a set of cultures was treated for approximately 20 hours without metabolic activation and harvested approximately 20 hours from the initiation of treatment. Replicate cultures were used at each concentration (19.0, 27.1, 38.8, 55.4, 79.1, 113, 161, 231, 329, 471, 672, 960, 1370, 1960 and 2800 µg/ml) with and without metabolic activation, for the negative and vehicle controls, and for each of the two concentrations of the positive control substances. Cultures treated with 960, 1370, 1960 and 2800 µg/ml without

metabolic activation (3- and 20-hour treatments) and with metabolic activation were analysed for chromosomal aberrations. After slide preparation and staining of the cells, 200 metaphases per dose and treatment condition were analysed for chromosomal aberrations.

All dose levels tested with and without metabolic activation were non-toxic. No significant increase in cells with chromosomal aberrations, polyploidy or endoreduplication was observed in the cultures analysed. The positive and vehicle controls fulfilled the requirements for a valid test. Under the conditions of this study, *N*-acetyl-glyphosate was concluded to be negative for the induction of structural and numerical chromosomal aberrations in cultured CHO cells with and without an exogenous metabolic activation system (Murli, 2004).

In a mammalian erythrocyte micronucleus test conducted according to OECD Test Guideline 474, groups of male and female Crl:CD1(ICR) mice received *N*-acetyl-glyphosate, sodium salt (purity 81.8%), dissolved in water at a single oral (gavage) dose of 500, 1000 or 2000 mg/kg bw in a volume of 10 ml/kg bw. A correction factor of 0.63 was used to derive the free acid equivalent from the weight of the test substance. Concurrent control groups were administered water (vehicle control) or cyclophosphamide at 30 mg/kg bw (positive control). The vehicle control and the low-dose and intermediate-dose groups contained 10 animals of each sex, the high-dose group contained 14 animals of each sex and the positive control group consisted of 5 animals of each sex. Half of the animals in each test substance and vehicle control group were sacrificed at each time point, approximately 24 and 48 hours post-dosing, respectively. The positive control group was sacrificed approximately 24 hours post-dosing. Bone marrow smears were prepared immediately after the sacrifices, 2000 polychromatic erythrocytes (PCEs) per animal were evaluated for micronuclei and 1000 total erythrocytes per animal were evaluated for bone marrow toxicity.

No clinical signs of toxicity or mortality were observed in the range-finding experiment at a single oral dose of 1500 or 2000 mg/kg bw. In the main study, there were no significant changes in body weight or body weight gain in either male or female animals administered the test substance or in the vehicle or positive control groups. No clinical signs of toxicity were observed at any dose level in male or female mice exposed to the test substance. No abnormalities were detected in the vehicle or positive control groups. No mortality occurred during the study.

No statistically significant increases in micronucleated PCE frequency were observed in any evaluated test substance–treated group of male or female animals at either time point. There were no statistically significant decreases in PCEs among 1000 erythrocytes. A statistically significant increase in micronucleated PCE frequency was found in positive control animals of both sexes. Under the conditions of this study, *N*-acetyl-glyphosate was considered not to be clastogenic or aneugenic in vivo in mice (Donner, 2006).

Genotoxicity studies with *N*-acetyl-glyphosate are summarized in Table 2.

2. *N*-Acetyl-AMPA (metabolite of glyphosate in genetically modified plants)

In an acute oral toxicity study conducted according to OECD Test Guideline 425 (Up-and-Down Procedure), three fasted female Crl:CD(SD) rats received *N*-acetyl-AMPA (purity 79%), suspended in deionized water, by oral gavage at a single dose of 5000 mg/kg bw; the dose volume was 20 ml/kg bw. The animals were dosed one at a time at a minimum of 48-hour intervals. Animals were observed for clinical signs of toxicity, body weight effects and mortality for 14 days after dosing. All animals were examined to detect grossly observable evidence of organ or tissue damage or dysfunction.

No mortalities were observed. Clinical signs of toxicity were observed in all rats and included diarrhoea, dark eyes, lethargy, high posture, stained fur/skin, wet fur, ataxia and/or hyper-reactivity. All animals appeared normal by day 3 or earlier and throughout the remainder of the study. There was

Table 2. Summary of genotoxicity studies with N-acetyl-glyphosate

End-point	Test object	Concentration or dose	Purity (%)	Result	Reference
In vitro					
Reverse mutation	*Salmonella typhimurium* (TA98, TA100, TA1535, TA1537); *Escherichia coli* (WP2*uvr*A)	±S9 mix: 0–5000 µg/plate	84.3	Negative	Mecchi (2004)
Gene mutation, *HPRT* locus	CHO cells	±S9 mix: 0–2091 µg/ml	81.8	Negative	Glatt (2006)
Chromosomal aberration	CHO cells	±S9 mix: 0–2800 µg/ml	84.3	Negative	Murli (2004)
In vivo					
Micronucleus induction	Male and female Crl:CD1(ICR) mouse bone marrow erythroblasts	0, 500, 1000 and 2000 mg/kg bw; single oral (gavage) administration	81.8	Negative	Donner (2006)

CHO, Chinese hamster ovary; S9, 9000 × *g* rat liver supernatant

no effect on body weights, and no test substance–related gross lesions were observed at necropsy. Under the conditions of this study, the oral LD_{50} for *N*-acetyl-AMPA was greater than 5000 mg/kg bw for female rats (Carpenter, 2007).

In a reverse gene mutation assay in bacteria conducted according to OECD Test Guideline 471, *Salmonella typhimurium* (strains TA98, TA100, TA1535 and TA1537) and *Escherichia coli* (strain WP2*uvr*A) were exposed to *N*-acetyl-AMPA (purity 76%), using deionized water as solvent, in the presence or absence of S9 metabolic activation. The dosing solutions were adjusted to compensate for the purity of the test substance using a correction factor of 1.45. In the initial toxicity–mutation test, dose levels of 1.5, 5.0, 15, 50, 150, 500, 1500 and 5000 µg/plate were evaluated using standard plate incorporation methods. In the confirmatory mutagenicity test, dose levels of 50, 150, 500, 1500 and 5000 µg/plate were evaluated. The highest dose level was set based on findings of the initial toxicity–mutation test and the guideline limit dose for this test system. The test substance was administered to the test system as a solution in water at a concentration of 50 mg/ml. Neither precipitate nor appreciable toxicity was observed. The positive and solvent controls fulfilled the requirements for a valid test.

The number of revertants at all concentrations of the test substance was similar to that for concurrent controls in trials both with and without activation. Under the conditions of this study, *N*-acetyl-AMPA was negative for mutagenic activity in non-activated and S9-activated bacterial test systems (Wagner & Klug, 2007).

In an in vitro mammalian cell gene mutation test conducted according to OECD Test Guideline 476, *N*-acetyl-AMPA (purity 72%) dissolved in water was tested for its ability to induce forward mutations at the *HPRT* locus in CHO cells. Two independent sets of experiments were conducted in the presence and absence of S9 metabolic activation. The dosing solutions were adjusted to compensate for the purity of the test substance using a correction factor of 1.389. Based on the results from a preliminary cytotoxicity assay, concentrations of 100–1531 µg/ml were used in the mutagenesis assay both with and without metabolic activation, and the same concentrations were used for the independent repeats. EMS and BaP served as positive controls in the experiments without and with metabolic activation, respectively. The cells were treated for 5 hours in both experiments, without and with metabolic activation. At the end of the exposure period, cells were washed and subcultured at 2- to 3-day intervals for a 7-day expression period. This was followed by incubation of the cells for 10 days in selection medium containing 6-thioguanine.

No visible precipitate in the treatment medium and no substantial toxicity were observed at any concentration in either non-activated or activated test systems. In trial 1, a high spontaneous mutant frequency was observed in both test conditions, resulting in an invalid test. The test was repeated (trial 2) with the same concentrations, and no positive responses were observed. The positive control substances EMS and BaP resulted in a marked increase in mutant frequency. Under the conditions of this study, *N*-acetyl-AMPA was considered to be not mutagenic in the CHO/HPRT forward mutation assay, in either the presence or absence of metabolic activation (Glatt, 2007).

In an in vitro mammalian chromosomal aberration test conducted according to OECD Test Guideline 473, the clastogenic potential of *N*-acetyl-AMPA (purity 76%), using deionized water as solvent, was tested in human peripheral blood lymphocytes in the presence or absence of S9 metabolic activation. The dosing solutions were adjusted to compensate for the purity of the test substance using a correction factor of 1.45. A vehicle control and positive controls (cyclophosphamide and mitomycin C for the tests with and without metabolic activation, respectively) were included to demonstrate the sensitivity of the test system. In the preliminary toxicity assay, the maximum concentration tested was 1530 µg/ml (10 mmol/l). In the chromosomal aberration assay, the concentrations tested were 191.25, 382.5, 765 and 1530 µg/ml, and the upper three concentrations were selected for evaluation. Cells were treated for 4 and 20 hours (non-activated test system) and 4 hours (activated test system). After exposure to colcemid, metaphase cells were harvested 20 hours following the initiation of treatment. Cells were evaluated for toxicity (mitotic inhibition), then structural and numerical chromosomal aberrations. After slide preparation and staining of the cells, 200 metaphases per dose and treatment condition were analysed for chromosomal aberrations.

There was no precipitation of the test substance in the treatment medium, and no substantial toxicity was observed at any concentration in all treatment groups. There were no statistically significant increases in structural chromosomal aberrations or in polyploidy at any of the concentrations evaluated. Positive controls induced the appropriate response. Under the conditions of this study, *N*-acetyl-AMPA was concluded to be negative for the induction of structural and numerical chromosomal aberrations in both the non-activated and S9-activated test systems in the in vitro mammalian chromosomal aberration test using human peripheral blood lymphocytes (Gudi & Rao, 2007).

In a mammalian erythrocyte micronucleus test conducted according to OECD Test Guideline 474, groups of male and female Crl:CD1(ICR) mice received *N*-acetyl-AMPA (purity 72%) dissolved in water at a single oral (gavage) dose of 500, 1000 or 2000 mg/kg bw in a volume of 10 ml/kg bw. A correction factor based on the percentage of active ingredient was used for preparation of the dosing solutions. Concurrent control groups were administered water (vehicle control) or cyclophosphamide (positive control) at 30 mg/kg bw. The vehicle control and the low-lose and intermediate-dose groups contained 10 animals of each sex, the high-dose group contained 14 animals of each sex and the positive control group consisted of 5 animals of each sex. Half of the animals in each test substance and vehicle control group were sacrificed at each time point, approximately 24 and 48 hours post-dosing, respectively. The positive control group was sacrificed approximately 24 hours post-dosing. Bone marrow smears were prepared immediately after the sacrifices; 2000 PCEs per animal were evaluated for micronuclei, and 1000 total erythrocytes per animal were evaluated for bone marrow toxicity.

No clinical signs of toxicity or mortality were observed in the range-finding experiment at a single oral dose of 2000 mg/kg bw. In the main study, there were no significant changes in body weight or body weight gain in either male or female animals administered the test substance or in the vehicle or positive control groups. No clinical signs of toxicity were observed at any dose level in male or female mice exposed to the test substance. No abnormalities were detected in the vehicle or positive control groups. No mortality occurred during the study.

Table 3. Summary of genotoxicity studies with N-acetyl-AMPA

End-point	Test object	Concentration or dose	Purity (%)	Result	Reference
In vitro					
Reverse mutation	*Salmonella typhimurium* (TA98, TA100, TA1535, TA1537); *Escherichia coli* (WP2*uvr*A)	±S9 mix: 0–5000 µg/plate	76	Negative	Wagner & Klug (2007)
Gene mutation, *HPRT* locus	CHO cells	±S9 mix: 0–1531 µg/ml	72	Negative	Glatt (2007)
Chromosomal aberration	Human peripheral blood lymphocytes	±S9 mix: 0–1530 µg/ml	76	Negative	Gudi & Rao (2007)
In vivo					
Micronucleus induction	Male and female Crl:CD1(ICR) mouse bone marrow erythroblasts	0, 500, 1000 and 2000 mg/kg bw; single oral (gavage) administration	72	Negative	Donner (2007)

CHO, Chinese hamster ovary; S9, 9000 × *g* rat liver supernatant

No statistically significant increases in micronucleated PCE frequency were observed in any evaluated test substance–treated group of male or female animals at either time point. There were no statistically significant decreases in PCEs among 1000 erythrocytes. A statistically significant increase in micronucleated PCE frequency was found in positive control animals of both sexes. Under the conditions of this study, *N*-acetyl-AMPA was considered not to be clastogenic or aneugenic in vivo in mice (Donner, 2007).

Genotoxicity studies with *N*-acetyl-AMPA are summarized in Table 3.

3. Comparison of toxicological properties of glyphosate and its plant metabolites, *N*-acetyl-glyphosate, AMPA and *N*-acetyl-AMPA

The comparison of key data on the toxicokinetics and metabolism of glyphosate and *N*-acetyl-glyphosate (Table 4) shows that both compounds have similar properties regarding the rate and extent of excretion, the lack of potential for accumulation and the very limited metabolism in animals. Oral absorption was approximately 2-fold higher for *N*-acetyl-glyphosate compared with glyphosate, but did not result in a higher acute or short-term toxicity (Table 5).

No study on toxicokinetics and metabolism has been performed with *N*-acetyl-AMPA. However, it was detected as a minor metabolite formed following oral administration of *N*-acetyl-glyphosate. It is not expected to be absorbed quickly from the gastrointestinal tract, as it is a charged molecule at physiological pH.

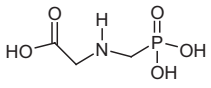
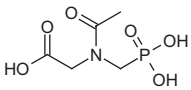
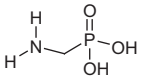
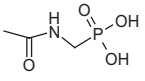
For *N*-acetyl-glyphosate, the available toxicological data package is almost complete except for studies on reproductive toxicity and long-term toxicity and carcinogenicity. A comparison with the toxicological studies performed with glyphosate showed that *N*-acetyl-glyphosate is of no greater toxicity than glyphosate (Table 5). In particular, despite a similar NOAEL for *N*-acetyl-glyphosate and glyphosate in short-term toxicity assays, the only effect occurring with *N*-acetyl-glyphosate was a slightly reduced body weight gain in male rats at a high dose of 1157 mg/kg bw per day, whereas no effects were observed in females at doses up to 1461 mg/kg bw per day. With regard to reproductive toxicity potential and carcinogenicity, it is not expected that *N*-acetyl-glyphosate can cause such effects, also taking into account chemical structural similarity with glyphosate. This assumption is supported by a structure–activity relationship (SAR) analysis of *N*-acetyl-glyphosate, with a lack of structural alerts for carcinogenicity, mutagenicity and endocrine effects.

Table 4. Comparison of toxicokinetics and metabolism of glyphosate and N-acetyl-glyphosate

	Glyphosate	*N*-Acetyl-glyphosate
Rate and extent of absorption	Rapid, approximately 30–36%	Rapid, approximately 66%
Distribution	Widely distributed	No information available
Potential for accumulation	No evidence, < 1% after 7 days (< 0.4% in residual carcass)	No evidence, 0.23% in residual carcass after 7 days
Rate and extent of excretion	Largely complete within 48 h, about 30% in urine and 70% in faeces	More than 90% within 48 h, about 66% in urine and 26% in faeces
Metabolism in rats	Very limited (< 0.7%), one metabolite (AMPA) detected in urine	Very limited (< 1%), two metabolites (glyphosate, N-acetyl-AMPA) detected in urine
Toxicologically significant compounds	Parent compound, AMPA	Parent compound, glyphosate

From Cheng & Howard (2004); Annex 1, reference *103*; O'Neal (2010)

Table 5. Summary of toxicological data for glyphosate, N-acetyl-glyphosate, AMPA and N-acetyl-AMPA

	Glyphosate	*N*-Acetyl-glyphosate	AMPA	*N*-Acetyl-AMPA
Chemical structure				
Acute oral toxicity, rat, LD_{50}	> 5000 mg/kg bw	> 5000 mg/kg bw	> 5000 mg/kg bw	> 5000 mg/kg bw
90-day oral toxicity, rat				No study submitted
- NOAEL	300 mg/kg bw	283 mg/kg bw	1000 mg/kg bw	
- LOAEL	811 mg/kg bw	1157 mg/kg bw	> 1000 mg/kg bw	
- critical effect	Salivary gland changes	Decreased body weight gain	No effects observed	
Gene mutation (Ames)	Negative	Negative	Negative	Negative
Gene mutation (*HPRT*)	Negative	Negative	Negative	Negative
Chromosomal aberration	Negative	Negative	Negative	Negative
Micronucleus test, in vivo	Negative	Negative	Negative	Negative

LD_{50}, median lethal dose; LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level
Source of data on glyphosate and AMPA: Annex 1, reference *103*

For *N*-acetyl-AMPA, the available toxicological data package is limited (there are only an acute toxicity study and a complete genotoxicity data package), but a wide database is available for the structurally similar metabolite, AMPA. Comparison of toxicological studies among *N*-acetyl-AMPA, AMPA and glyphosate leads to the conclusion that *N*-acetyl-AMPA is of no greater toxicity than glyphosate (Table 5). This assumption is supported by a SAR analysis of *N*-acetyl-AMPA, with a lack of structural alerts for carcinogenicity, mutagenicity and endocrine effects.

Comments

Biochemical aspects

[^{14}C]*N*-Acetyl-glyphosate was rapidly and incompletely (approximately 66%) absorbed in rats following a single oral dose of 15 mg/kg bw. The maximum concentration of radioactivity in plasma was reached after 2 hours, and the half-life for elimination from plasma was 15.6 hours. Elimination was mainly via urine (66.1%) and, to a lesser extent, faeces (26.4%); more than 90% of the total radi-

oactivity was eliminated by 48 hours post-dosing. *N*-Acetyl-glyphosate was metabolized to a very limited extent. One metabolite, glyphosate (< 1% of the total radioactivity), was detected in faeces after a single oral dose of 15 mg/kg bw, whereas glyphosate and *N*-acetyl-AMPA were found in urine following subchronic exposure at dose levels of 56 mg/kg bw per day and above.

Toxicological data

N-Acetyl-glyphosate

N-Acetyl-glyphosate was of low acute oral toxicity; the LD_{50} was greater than 5000 mg/kg bw in rats.

In a 90-day study of toxicity with *N*-acetyl-glyphosate in rats, the NOAEL was 4500 ppm (equal to 283 mg/kg bw per day), based on slightly decreased body weight gains in male rats at 18 000 ppm (equal to 1157 mg/kg bw per day).

N-Acetyl-glyphosate was tested for genotoxicity in vitro and in vivo in an adequate range of assays; it was not found to be genotoxic in mammalian and microbial test systems.

The Meeting concluded that *N*-acetyl-glyphosate was unlikely to be genotoxic.

The Meeting concluded that *N*-acetyl-glyphosate is of no greater toxicological concern than the parent glyphosate, based on the structural similarity of *N*-acetyl-glyphosate with glyphosate and supported by the following considerations: 1) *N*-acetylation is a common detoxification pathway of xenobiotic compounds in mammals; therefore, *N*-acetyl-glyphosate is expected to be of similar toxicity to or lower toxicity than glyphosate; 2) a SAR analysis indicates that the *N*-acetylated group is not a structural alert for carcinogenicity, mutagenicity or endocrine effects; and 3) the toxicological data for *N*-acetyl-glyphosate show low acute toxicity, low subchronic toxicity (with no organ toxicity in rats at doses up to 1157 mg/kg bw per day) and a lack of genotoxicity.

N-Acetyl-AMPA

N-Acetyl-AMPA was of low acute oral toxicity; the LD_{50} was greater than 5000 mg/kg bw in rats.

N-Acetyl-AMPA was tested for genotoxicity in vitro and in vivo in an adequate range of assays; it was not found to be genotoxic in mammalian or microbial test systems.

The Meeting concluded that *N*-acetyl-AMPA was unlikely to be genotoxic.

The Meeting concluded that the toxicity of *N*-acetyl-AMPA is low and of limited concern, based on the structural similarity of *N*-acetyl-AMPA with AMPA and supported by the following considerations: 1) *N*-acetyl-AMPA is a charged molecule at physiological pH and is expected to be poorly absorbed from the gastrointestinal tract; 2) *N*-acetylation is a common detoxification pathway of xenobiotic compounds in mammals; therefore, *N*-acetyl-AMPA is expected to be of similar toxicity to or lower toxicity than AMPA or glyphosate; and 3) a SAR analysis indicates that the *N*-acetylated group is not a structural alert for carcinogenicity, mutagenicity or endocrine effects.

Toxicological evaluation

The Meeting concluded that the group ADI of 0–1 mg/kg bw established by the 2004 JMPR for glyphosate and AMPA may also be applied to *N*-acetyl-glyphosate and *N*-acetyl-AMPA, as the available toxicological data showed that these plant metabolites have no greater toxicity than the parent glyphosate.

The 2004 JMPR decided that an ARfD for glyphosate was unnecessary. The present Meeting confirmed that it is not necessary to establish an ARfD for *N*-acetyl-glyphosate or *N*-acetyl-AMPA

in view of their low acute toxicity and the absence of any toxicological effects that would be likely to be elicited by a single dose.

Estimate of acceptable daily intake for humans

0–1 mg/kg bw (for the sum of glyphosate, *N*-acetyl-glyphosate, AMPA and *N*-acetyl-AMPA)

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

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[1] Study sponsored by Pioneer Hi-Bred, which is a company owned by E.I. du Pont de Nemours and Company.

[2] Study sponsored by Verdia, Inc. Data purchased from Verdia by E.I. du Pont de Nemours and Company.

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[1] Study sponsored by Verdia, Inc. Data purchased from Verdia by E.I. du Pont de Nemours and Company.

ISOPYRAZAM

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Explanation

Isopyrazam is the provisional International Organization for Standardization (ISO)–approved name for a mixture of two *syn* isomers of 3-(difluoromethyl)-1-methyl-*N*-[(1*RS*,4*SR*,9*RS*)-1,2,3,4-tetrahydro-9-isopropyl-1,4-methanonaphthalen-5-yl]pyrazole-4-carboxamide and two *anti* isomers

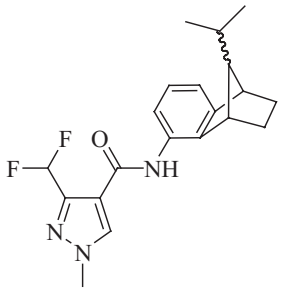
of 3-(difluoromethyl)-1-methyl-*N*-[(1*RS*,4*SR*,9*SR*)-1,2,3,4-tetrahydro-9-isopropyl-1,4-methanonaphthalen-5-yl]pyrazole-4-carboxamide (Chemical Abstracts Service No. 881685-58-1). Technical isopyrazam contains *syn* (SYN534969) and *anti* (SYN534968) isomers at ratios from 70:30 to 100:0 *syn*:*anti*.

Isopyrazam is a new broad-spectrum fungicide of the *ortho*-substituted phenyl amides, acting by inhibition of succinate dehydrogenase. It 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 Forty-second Session of the Codex Committee on Pesticide Residues.

All the pivotal studies met the requirements of the relevant Organisation for Economic Co-operation and Development or national test guidelines and were certified as complying with good laboratory practice.

The chemical structure of isopyrazam is shown in Figure 1.

Figure 1. Chemical structure of isopyrazam



Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

A whole-body autoradiography and expired air study in male and female Han Wistar rats was performed. Single doses of 97.8% radiochemically pure [pyrazole-5-^{14}C]isopyrazam (labelled 92.25:7.75 *syn*:*anti*; non-labelled 92.8:7.2 *syn*:*anti*) at 2.5 mg/kg body weight (bw) (3.73 MBq/mg) or 250 mg/kg bw (0.0396 MBq/mg) were administered by oral gavage to one animal of each sex in the expired air study group and to four animals of each sex in the whole-body autoradiography study group. The radioactivity in expired air, urine, faeces and cage wash was determined at 10, 24, 34 (air only) and 48 hours after dosing. The radioactivity remaining in the blood, plasma, gastrointestinal tract plus contents and residual carcass of each rat was also determined. In the whole-body autoradiography group, subgroups of one male and one female rat were terminated at 2, 24 and 48 hours after dosing, and the distribution of radioactivity in the carcass was determined by whole-body autoradiography.

The recovery of radioactivity was 96–98% in the 2.5 mg/kg bw groups and 76–87% in the 250 mg/kg bw groups. After 48 hours, the total radioactive residues (TRR) in expired air were less than 0.05% of the dose in both male and female rats at both dose levels. At both dose levels, 2 hours after dosing, the majority of the radioactivity was found in the stomach, liver, gastrointestinal tract and kidney. Lower concentrations were found in the Harderian gland, abdominal fat and brown fat. Low levels of radioactivity were seen in heart, lungs, spleen, pancreas, adrenals, thymus, salivary glands, testes, ovaries, uterus, brain, spinal cord and muscle. Radioactivity was not seen in the thyroid, eye

or bone. By 24 hours after dosing, very little radioactivity remained in the bodies of the rats. TRR depletion was slower in high-dose females than in low-dose females. The majority of the remaining radioactivity was found in the gastrointestinal tract and stomach, with lower levels in the liver and kidney (Silcock, 2007).

To investigate the pharmacokinetics of isopyrazam, single doses of 98.6% radiochemically pure [pyrazole-5-^{14}C]isopyrazam (labelled 98.8:1.2 *syn*:*anti*; non-labelled 92.8:7.2 *syn*:*anti*) at 1 mg/kg bw (5.03 MBq/mg) or 75 mg/kg bw (0.07 MBq/mg) were administered by oral gavage to 13 Han Wistar rats of each sex per dose. For pharmacokinetic analyses in blood and plasma, nine animals of each sex per dose group were allocated to three blood sampling groups covering 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 12, 24 and 48 hours after dosing. To obtain plasma for metabolic profiling, two animals of each sex per dose were terminated at 3 and 6 hours after dosing, and plasma was analysed in a separate study (Green, 2008).

Maximum blood and plasma TRR concentrations were achieved at 3–6 hours in male and female rats after dosing of 1 or 75 mg/kg bw (Table 1). The mean terminal half-lives in blood and plasma of low-dose females were similar (4.6 and 4.8 hours). In males, terminal half-life and area under the curve ($AUC_{0-\infty}$) values could not be calculated because no terminal mono-exponential phase could be identified. For the same reason, the half-life and $AUC_{0-\infty}$ in plasma of high-dose females could not be calculated. However, excretion was virtually complete within 48 hours (Figures 2 and 3). In plasma and blood at both dose levels, females had higher TRR concentrations than males. Radioactivity was excreted via the faeces, accounting for 70.9% and 78.0% at the two dose levels, respectively. Excretion via the urine was 12.9% and 18.0% of the administered dose at the two dose levels, respectively (Allan, 2008a).

To investigate the excretion pattern and tissue distribution of isopyrazam, single doses of 99.4% radiochemically pure [pyrazole-5-^{14}C]isopyrazam (labelled 98.8:1.2 *syn*:*anti*; non-labelled 92.8:7.2 *syn*:*anti*) at 1 mg/kg bw (4.88 MBq/mg) and 75 mg/kg bw (0.06 MBq/mg) were administered by oral gavage to four Han Wistar rats of each sex per dose. The age of the high-dose animals was 6–7 weeks, and that of the low-dose animals was 13 weeks. The excreta of the high-dose animals were also used in another study (Allan, 2008a). Urine, faeces and cage washes were collected over 7 days, and animals were then killed. TRR in the organs were analysed.

Recovery of TRR was 106.0–106.4% in the low-dose group and 95.7–100.5% in the high-dose group. In the low-dose group, excretion was virtually complete within 48 hours; 77.3–83.0% of TRR was recovered in faeces, and 19.6–27.1% in urine. In the high-dose group, 89.8–92.2% of the administered dose was excreted within 48 hours. At 7 days post-dosing, 78.5–79.4% was excreted via faeces and 13.3–17.5% via urine. At day 7 post-dosing, animals of both sexes in the low-dose group had 0.005–0.010 µg equivalents (eq) per gram in the liver and 0.002–0.003 µg eq/g in the kidney. The concentration of TRR in all other organs was below the limit of detection. In the high-dose group, animals of both sexes had 0.284–0.586 µg eq/g in the liver, and a concentration of 0.121 µg eq/g was measured in the kidneys of males. The concentration of TRR in all other organs was below the limit of detection (tissue limit of detection not stated) (Shaw, 2008b).

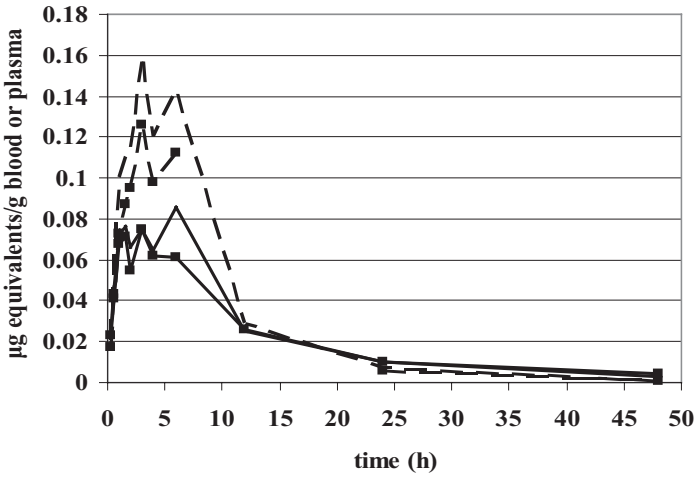
To investigate the tissue depletion of isopyrazam from tissues, single doses of 99.4% radiochemically pure [pyrazole-5-^{14}C]isopyrazam (labelled 98.8:1.2 *syn*:*anti*; non-labelled 92.8:7.2 *syn*:*anti*) at 1 mg/kg bw (4.88 MBq/mg) or 75 mg/kg bw (0.06 MBq/mg) were administered by oral gavage to 15 Han Wistar rats of each sex per dose. At 6, 12, 24, 48 and 96 hours post-dosing in the low-dose group and 10, 18, 24, 48 and 96 hours post-dosing in the high-dose group, three animals of each sex per dose were killed, and organs, tissues and body fluids were retained for TRR analysis.

Table 1. Plasma and blood pharmacokinetic parameters in rats following single oral administration of isopyrazam at 1 or 75 mg/kg bw

	1 mg/kg bw				75 mg/kg bw			
	Blood		Plasma		Blood		Plasma	
	Males	Females	Males	Females	Males	Females	Males	Females
C_{max} (µg eq/ml)	0.075	0.126	0.0857	0.160	6.31	12.2	7.56	17.7
T_{max} (h)	3	3	6	3	3	4	3	4
AUC_{0-t} (µg eq·h/ml)	1.00	1.62	1.14	1.44	96.9	210	81.4	207
$AUC_{0-\infty}$ (µg eq·h/ml)	NC	1.67	NC	1.49	98.7	211	82.7	NC
$t_{½}$ (h)	NC	4.81	NC	4.60	8.68	6.21	7.52	NC

From Allan (2008a)
AUC, area under the concentration–time curve; C_{max}, peak concentration; eq, equivalents; NC, not calculated; $t_{½}$, half-life; T_{max}, time to reach C_{max}

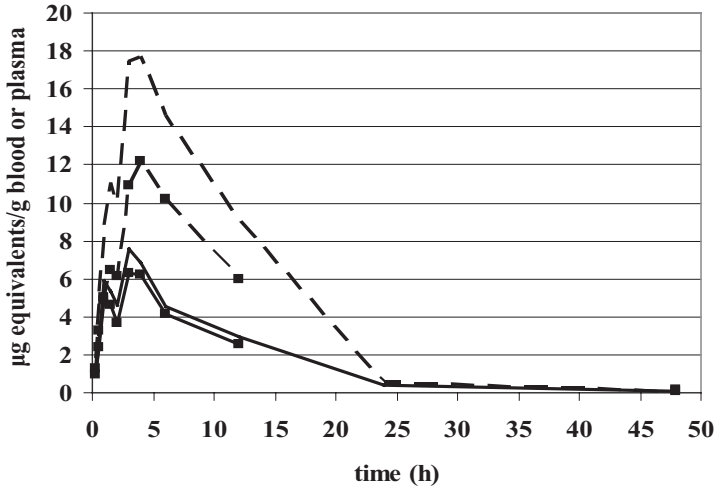
Figure 2. Mean total radioactive residue concentrations in plasma and blood of male and female rats following a single oral administration of [pyrazole-5-^{14}C]isopyrazam at 1 mg/kg bw



Solid line (males), dashed line (females), squares (blood), no symbols (plasma)
Source: Allan (2008a)

At 1 mg/kg bw, the highest levels of TRR were observed in both sexes at the first sampling time at 6 hours. In males at this time point, 3.1% of the dose was found in the carcass and 2.3% in liver; in females, the respective values were 7.1% and 2.6%. In males, the mean concentrations were 0.551 µg eq/g in the liver, 0.310 µg eq/g in the kidney and 0.196 µg eq/g in the adrenals. In females, the respective values were 0.677 µg eq/g, 0.397 µg eq/g and 0.356 µg eq/g. In both sexes, the concentration of TRR in tissues declined, retaining the relative organ distribution, and the initially higher TRR contents in female organs converged to the concentrations in males. At 96 hours post-dosing, the TRR remaining in the carcass accounted for approximately 0.5% of the dose. In male rats, the elimination half-lives for blood, heart, kidneys, liver and lungs were similar and were in the range of 29–53 hours. The elimination half-lives for plasma and bone mineral were 11 and 87 hours, respectively. In female rats, the elimination half-lives for bone mineral, brain and plasma were very similar and were in the range of 3–5 hours. The elimination half-lives for thymus, uterus, lungs, liver, renal fat, pancreas and kidneys were 10, 14, 23, 24, 53, 67 and 71 hours, respectively.

Figure 3. Mean total radioactive residue concentrations in plasma and blood of male and female rats following a single oral administration of [pyrazole-5-^{14}C]isopyrazam at 75 mg/kg bw



Solid line (males), dashed line (females), squares (blood), no symbols (plasma)
Source: Allan (2008a)

At 75 mg/kg bw, the highest levels of TRR were observed in both sexes at the first sampling time at 10 hours. In males at this time point, 6.9% of the dose was found in the carcass and 2.5% in liver; in females, the respective values were 8.2% and 1.8%. In males, the mean concentrations were 53.5 µg eq/g in the liver, 17.0 µg eq/g in the kidney, 16.1 µg eq/g in the thyroid, 14.1 µg eq/g in the bone mineral and 13.8 µg eq/g in the adrenals. In females, 58.0 µg eq/g were found in the renal fat, 35.5 µg eq/g in the liver, 23.7 µg eq/g in the ovaries, 28.2 µg eq/g in the adrenals and 20.2 µg eq/g in the uterus. TRR levels in pancreas, kidneys, lungs, thyroid, heart and spleen were also higher than those observed in whole blood (6.13 µg eq/g). In both sexes, TRR levels in tissues declined, retaining the relative organ distribution. At 96 hours, detectable TRR levels in males and females were found in liver and renal fat and in males also in kidney, pancreas and lungs. In males, the remaining radioactivity accounted for 0.48% of the administered dose, and in females, it was barely quantifiable. In male rats, the elimination half-lives for adrenals, heart, muscle, plasma, spleen, testes and thymus were very similar and were in the range of 4–6 hours. The elimination half-lives for blood, lungs, renal fat and kidneys were 8, 24, 48 and 49 hours, respectively. In female rats, the elimination half-lives for adrenals, lungs, ovaries, plasma, spleen and thymus were very similar and were in the range of 3–5 hours. The elimination half-lives for muscle, pancreas, uterus and blood were also very similar and were in the range of 6–7 hours. The elimination half-lives for renal fat and liver were 17 and 29 hours, respectively (Allan, 2008b).

To investigate the excretion and tissue distribution of isopyrazam, 30 male Han Wistar rats were administered by gavage daily doses of 1 mg/kg bw per day (0.954 MBq/mg) of 99.4% radiochemically pure [pyrazole-5-^{14}C]isopyrazam (labelled 98.8:1.2 *syn*:*anti*; non-labelled 92.8:7.2 *syn*:*anti*) for maximally 14 days. Twenty-four hours after 3, 7, 10 and 14 doses and 3, 7, 10, 14, 28 and 43 days after 14 doses, three animals per time point were killed. To analyse for TRR, organs and blood samples were collected. Additionally, for 24 hours after the first dose or for 24 hours after 14 doses, faeces and urine were collected.

The highest TRR levels were found in liver (0.361 µg eq/g), kidneys (0.121 µg eq/g) and blood (0.033 µg eq/g) at 24 hours after 14, 10 and 14 days, respectively. In most other organs, concentrations were below 0.05 µg eq/g. With the exception of the liver and renal fat, the steady-state TRR concentration in tissues was reached by 24 hours after dosing day 10. After cessation of dosing, the TRR concentration in organs decreased rapidly, and 14 days after dosing day 14, radioactivity was measurable only in liver and kidneys. In a 24-hour period after 1 day of exposure, 47.9% of the administered dose was excreted in faeces and 19.7% in urine. After a 14-day exposure period, 88.6% was excreted in faeces and 21.6% in urine within a collection period of 24 hours (Allan, 2008c).

To investigate the possibly different excretion profiles of *syn* and *anti* isopyrazam, single doses of 2 or 75 mg/kg bw of *syn* or *anti* [pyrazole-5-^{14}C]isopyrazam were administered by oral gavage to four male and four female bile duct–cannulated Han Wistar rats. Unlabelled *syn* isomer (SYN534969) was 98.7% chemically pure, with a 99.3:0.7 *syn*:*anti* isomer ratio; the corresponding radiolabelled *syn* isomer was 98.4% radiochemically pure. Unlabelled *anti* isomer (SYN534968) was 98.4% chemically pure, with a 1:99 *syn*:*anti* isomer ratio; the corresponding radiolabelled *anti* isomer was 98.5% radiochemically pure (mean of two batches used). The low-dose *syn* isomer had a specific activity of 2.51 MBq/mg, and the low-dose *anti* isomer, 1.69 MBq/mg. The high doses of *syn* and *anti* isomers both had a specific activity of 0.068 MBq/mg. The female high-dose *syn* isomer group consisted of seven animals, and the male high-dose *anti* isomer group, five animals. Urine, bile, faeces and cage washes were collected at intervals over 3 days, after which time the rats were terminated. A terminal blood sample was removed, together with the gastrointestinal tract and residual carcass, and each sample taken was analysed for residual radioactivity.

Three high-dose *syn* isomer females were killed prior to study termination because of severe clinical signs observed in this group. Effects were attributed to the surgical procedure exacerbated by treatment, rather than to treatment with isopyrazam alone.

For the *anti* isomer, at 2 mg/kg bw, the major routes of elimination in both males and females were the bile (means of 38.3% and 56.1% of the administered dose in males and females, respectively) and the faeces (means of 32.5% and 28.3% of the administered dose in males and females, respectively), recovered over 3 days post-dosing (Table 2). Urinary excretion accounted for means of 22.1% and 12.2% of the dose in males and females, respectively. Absorption accounted for 60.6% of the dose in males and 68.6% of the dose in females, as calculated from the biliary and urinary excretion and TRR remaining in the carcass. At 75 mg/kg bw, the major routes of elimination in both males and females were the bile (means of 36.5% and 61.1% of the administered dose in males and females, respectively) and the faeces (means of 38.3% and 20.4% of the administered dose in males and females, respectively), recovered over 3 days post-dosing. Urinary excretion accounted for means of 16.3% and 15.9% of the dose in males and females, respectively. Absorption accounted for 53.0% of the dose in males and 77.2% of the dose in females, as calculated from the biliary and urinary excretion and TRR remaining in the carcass.

For the *syn* isomer, at 2 mg/kg bw, the major routes of elimination in both males and females were the bile (means of 56.3% and 48.9% of the administered dose in males and females, respectively) and the faeces (means of 23.8% and 19.8% of the administered dose in males and females, respectively), recovered over 3 days post-dosing. Urinary excretion accounted for means of 15.4% and 26.0% of the dose in males and females, respectively. Absorption accounted for 71.8% of the dose in males and 75.1% of the dose in females, as calculated from the biliary and urinary excretion and TRR remaining in the carcass. At 75 mg/kg bw, the major routes of elimination in both males and females were the bile (means of 58.0% and 41.6% of the administered dose in males and females, respectively) and the faeces (means of 34.1% and 48.3% of the administered dose in males and females, respectively), recovered over 3 days post-dosing. Urinary excretion accounted for means of 7.6% and 7.0% of the dose in males and females, respectively. Absorption accounted for 65.7% of the

Table 2. Excretion of syn and anti isomers of isopyrazam in male and female rats at single doses of 2 and 75 mg/kg bw

Excreta	Excretion (% of administered dose)			
	2 mg/kg bw		75 mg/kg bw	
	Males	Females	Males	Females
***anti* isomer**				
Bile	38.3	56.1	36.5	61.1
Faeces	32.5	28.3	38.3	20.4
Urine	22.1	12.2	16.3	15.9
Total absorbed	60.6	68.6	53.0	77.2
***syn* isomer**				
Bile	56.3	48.9	58.0	41.6
Faeces	23.8	19.8	34.1	48.3
Urine	15.4	26.0	7.6	7.0
Total absorbed	71.8	75.1	65.7	48.7

From Shaw (2008a)

dose in males and 48.7% of the dose in females, as calculated from the biliary and urinary excretion and TRR remaining in the carcass.

Syn and *anti* isomers at both dose levels in both sexes were excreted at 84.4–97.0% of the administered dose within 24 hours. At 72 hours post-dosing, mean blood and plasma concentrations were below the limit of reliable detection in both sexes in all four groups.

In conclusion, there were no significant differences in absorption and excretion route and rate between the two isomers. Additionally, there was no significant influence of sex or dose (Shaw, 2008a).

To investigate the excretion pattern and tissue distribution of isopyrazam, single doses of 1 mg/kg bw (4.88 MBq/mg) or 75 mg/kg bw (0.08 MBq/mg) of 99.4% radiochemically pure *syn* [pyrazole-5-^{14}C]isopyrazam (labelled 98.8:1.2 *syn*:*anti*; non-labelled 92.8:7.2 *syn*:*anti*) were administered by oral gavage to four bile duct–cannulated Han Wistar rats of each sex per dose. Urine, bile, faeces and cage washes were collected over 2 days, after which the rats were terminated. A terminal blood sample was removed, together with the gastrointestinal tract and residual carcass, and each sample taken was then analysed for TRR.

At 1 mg/kg bw, the major routes of elimination in both males and females were the bile (means of 57.9% and 47.6% of the administered dose in males and females, respectively) and the faeces (means of 26.4% and 35.7% of the administered dose in males and females, respectively), recovered over 2 days post-dosing. Urinary excretion accounted for means of 14.9% and 15.9% of the dose in males and females, respectively. Absorption accounted for 72.9% of the dose in males and 63.7% of the dose in females, as calculated from the biliary and urinary excretion and TRR remaining in the carcass. At 75 mg/kg bw, the major routes of elimination in both males and females were the bile (means of 54.7% and 57.0% of the administered dose in males and females, respectively) and the faeces (means of 27.3% and 21.2% of the administered dose in males and females, respectively), recovered over 2 days post-dosing. Urinary excretion accounted for means of 7.3% and 13.6% of the dose in males and females, respectively. Absorption accounted for 63.1% of the dose in males and 71.4% of the dose in females, as calculated from the biliary and urinary excretion and TRR remaining in the carcass. Irrespective of dose and sex, 91.0–97.1% of the administered dose was excreted

within 24 hours post-dosing. Mean blood and plasma concentrations were below the limit of reliable detection at 48 hours post-dosing in both dose groups (Shaw, 2008c).

1.2 Biotransformation

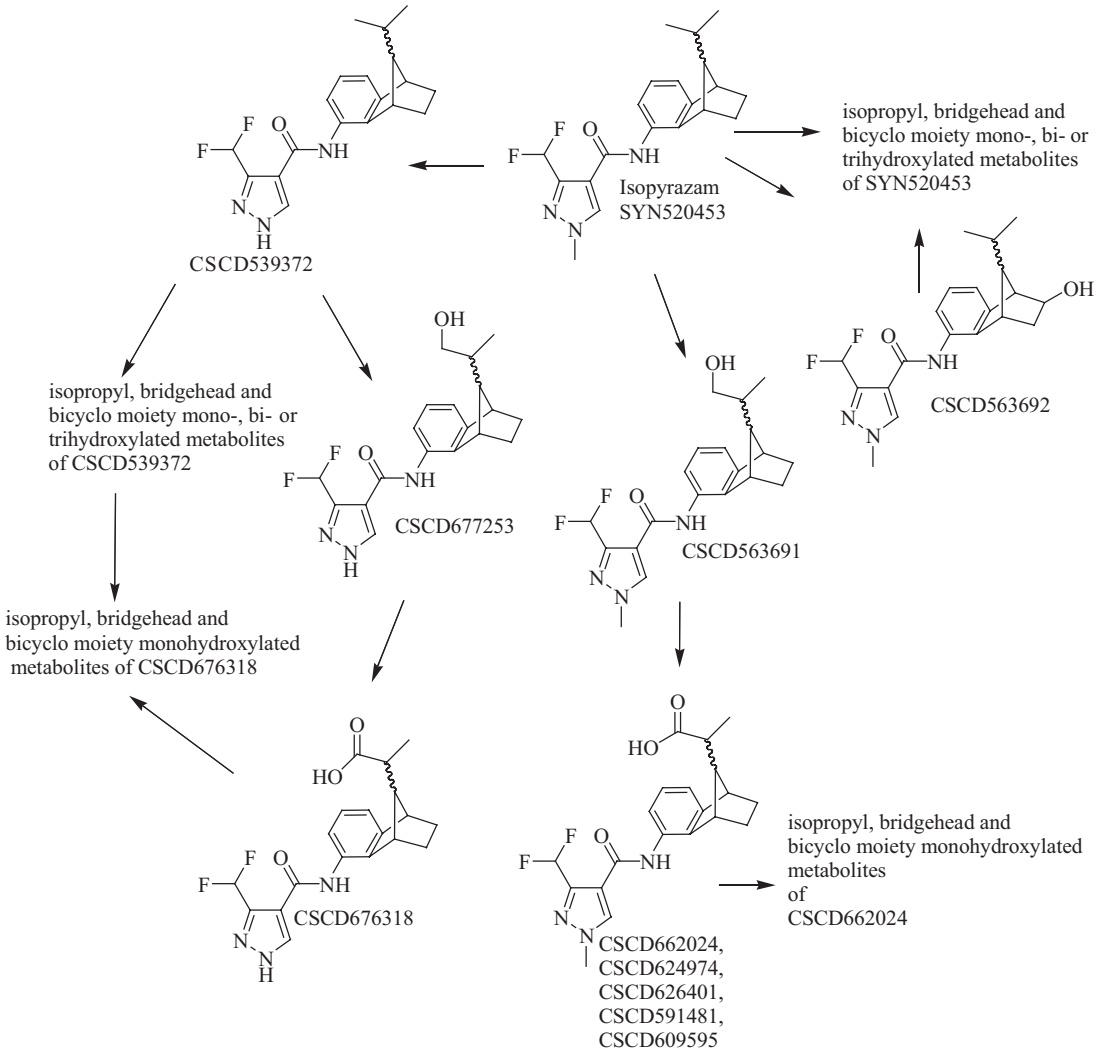
The metabolism of isopyrazam was investigated by analysing bile, faeces, urine and plasma samples retained from the previous studies: single oral administration of 1 or 75 mg/kg bw of *syn* [pyrazole-5-^{14}C]isopyrazam (Allan, 2008a; Shaw, 2008b,c), 14 oral doses of 1 mg/kg bw per day of *syn* [pyrazole-5-^{14}C]isopyrazam (Allan, 2008c), single oral administration of 2 or 75 mg/kg bw of *syn* [pyrazole-5-^{14}C]isopyrazam and single oral administration of 2 or 75 mg/kg bw of *anti* [pyrazole-5-^{14}C]isopyrazam (Shaw, 2008a).

The predominant metabolic pathway for isopyrazam is hydroxylation in the bicyclic isopropyl moiety, followed by further oxidation to form the carboxylic acid or to give rise to multiple hydroxyl moieties, with subsequent formation of glucuronic acid or sulfate conjugates (in Figure 4, the primary metabolism of isopyrazam is depicted; the sulfate and glucuronic acid conjugation products are not shown). Isopyrazam is also oxidized by *N*-demethylation, giving rise to the corresponding set of demethylated metabolites and their conjugates. The structure of isopyrazam provides the potential for stereoisomerization of most metabolites. For many metabolites, it was not possible to determine the exact position of hydroxylation or the conformation of the molecule. No cleavage of the molecule was observed. In general, metabolites excreted in urine tended to be unconjugated, comprising monohydroxy, dihydroxy, acid and hydroxy acids and their demethylated counterparts. In the urine of female rats, sulfate conjugates of these molecules were also detected. In bile, the glucuronide conjugates of the same oxidative metabolites were observed, along with a few metabolites in the unconjugated form. Male rat bile also contained small quantities of trihydroxylated metabolites, present as both conjugated and unconjugated entities. A similar range of metabolites was present in faeces. Some sulfate conjugates were detected in the faeces of female rats. There was less than 1% parent compound detected in the faeces of non-bile duct–cannulated rats. Isopyrazam was the only component detected in the faeces of bile duct–cannulated animals administered a high dose and was the major component in the faeces of bile duct–cannulated animals administered a low dose; the other components were identified as trihydroxy, dihydroxy and *N*-desmethyl dihydroxy metabolites. The metabolic profile of male plasma sampled at the time of the maximum plasma radioactivity concentration (T_{max}) showed the presence of two acid metabolites and three demethylated acid metabolites, with no parent compound present. In female T_{max} plasma samples, demethylated parent was the most abundant metabolite, with two *N*-desmethyl hydroxylated metabolites, an *N*-desmethyl dihydroxy metabolite, a sulfate conjugate of the *N*-desmethyl dihydroxy metabolite and an acid metabolite. Some parent compound was also detected. Given the high number of isopyrazam metabolites, each at low concentrations, sex and dosing regimen comparisons for metabolite profiles are difficult. Nevertheless, sex, dosing regimen and isomeric form of isopyrazam do not seem to influence the metabolism of isopyrazam (Green, 2008).

The in vitro metabolism of CSCD658108 (the *syn* isomer of CSCD563692; see Figure 4) and of SYN534969 (the *syn* isomer of SYN520453) by male Wistar rat liver microsomes was investigated. The compounds used were [pyrazole-5-^{14}C]CSCD658108 (specific activity 0.89 MBq/mg) and [pyrazole-5-^{14}C]SYN534969 (specific activity 2.54 MBq/mg). The compounds were incubated with microsomes at concentrations of 6.316–6.824 µmol/l for 15 minutes, and then metabolites were analysed.

CSCD658108 and SYN534969 were both metabolized to CSCD459488 and further to CSCD656800 (isopropyl and bicyclo dihydroxylated *syn* isopyrazam, also found in vivo in rats). Therefore, CSCD658108 also seems to be an in vivo precursor of CSCD656800 (White, 2008).

Figure 4. Metabolism of isopyrazam in the rat



1.3 Effects on enzymes and other biochemical parameters

No information was available.

2. Toxicological studies

2.1 Acute toxicity

The results of studies of acute toxicity with isopyrazam are summarized in Table 3.

(a) Oral administration

The acute oral toxicity of *syn* isopyrazam (batch No. SMU6BP001, purity 99.0%), *anti* isopyrazam (batch No. SMU6CP001, purity 95.5%) and isopyrazam (1:1 *syn*:*anti*, batch No. SMU6CP014, purity 98.2%) was tested in female HanRCC Wistar rats. The median lethal dose (LD_{50}) of pure *syn* isopyrazam in female rats was greater than 2000 mg/kg bw; the LD_{50} of both pure *anti* and 1:1 *syn*:*anti* isopyrazam was 310.2 mg/kg bw (95% confidence interval [CI] 175–550 mg/kg bw) (Ott, 2006a).

Table 3. Acute toxicity of isopyrazam

Diastereoisomer ratio	Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	LC_{50} (mg/l)	Purity (%)	Reference
syn	Rat	HanRCC:WIST	Female	Oral	> 2000	—	99.0	Ott (2006a)
anti	Rat	HanRCC:WIST	Female	Oral	310.2 (95% CI 175–550)	—	95.5	Ott (2006a)
syn:*anti* 1:1	Rat	HanRCC:WIST	Female	Oral	310.2 (95% CI 175–550)	—	98.2	Ott (2006a)
syn:*anti* 92.8:7.2	Rat	HanRCC:WIST	Female	Oral	> 2000	—	96.4	Arcelin (2007a)
syn:*anti* 69.7:30.3	Rat	HanRCC:WIST	Female	Oral	2000 (95% CI 864.4–4210)	—	90.8	Simon (2008c)
syn:*anti* 92.8:7.2	Rat	HanRCC:WIST	Male, female	Dermal	> 5000	—	96.45	Arcelin (2007b)
syn:*anti* 92.8:7.2	Rat	HsdBrlHan	Male, female	Respiratory	—	> 5.28	96.45	Rattray (2006a)

CI, confidence interval; LC_{50}, median lethal concentration; LD_{50}, median lethal dose

The acute oral toxicity of isopyrazam (92.8:7.2 *syn*:*anti*, batch No. SMU6AP001, purity 96.4%) was tested in female HanRCC Wistar rats. The LD_{50} of isopyrazam with a high *syn* epimer fraction in female rats was greater than 2000 mg/kg bw (Arcelin, 2007a).

The acute oral toxicity of isopyrazam (69.7:30.3 *syn*:*anti*, batch No. SMU7DP017, purity 90.8%) was tested in female HanRCC Wistar rats. The LD_{50} of isopyrazam with a two thirds *syn* epimer fraction in female rats was 2000 mg/kg bw (95% CI 864.4–4210 mg/kg bw). Treated animals showed clinical signs (ruffled fur > hunched posture > sedation) a few hours after dosing, which increased in severity with dose. All clinical signs were reversible by 3–8 days post-dosing (Simon, 2008c).

(b) Dermal application

The acute dermal toxicity of isopyrazam (92.8:7.2 *syn*:*anti*, batch No. SMU6AP001, purity 96.4%) was tested in male and female HanRCC Wistar rats. The dermal LD_{50} of isopyrazam with a high *syn* epimer fraction in male and female rats was greater than 5000 mg/kg bw (Arcelin, 2007b).

(c) Exposure by inhalation

The acute respiratory toxicity of isopyrazam (92.8:7.2 *syn*:*anti*, batch No. SMU6AP001, purity 96.45%) after a 4-hour nose-only exposure was tested in male and female HsdBrlHan rats. The respiratory median lethal concentration (LC_{50}) of isopyrazam with a high *syn* epimer fraction in male and female rats was greater than 5.28 mg/l (Rattray, 2006a).

(d) Dermal and ocular irritation

The primary skin irritation potential of 0.5 g isopyrazam per animal (92.8:7.2 *syn*:*anti*, batch No. SMU6AP001, purity 96.45%) was tested in male and female New Zealand White rabbits following exposure for 4 hours by semi-occlusive dressing. Isopyrazam with a high *syn* epimer fraction was not irritating to the skin in male and female rabbits (Ott, 2006b).

The primary eye irritation potential of 0.1 g isopyrazam per animal (92.8:7.2 *syn*:*anti*, batch No. SMU6AP001, purity 96.45%) was tested in male and female New Zealand White rabbits by instillation into the left eye of the animals. Isopyrazam with a high *syn* epimer fraction was only

initially slightly irritating to the conjunctivae (reddening and chemosis) in male and female rabbits. There were no other effects on the eye (Talvioja, 2007).

(e) Dermal sensitization

In a mouse local lymph node assay to investigate the skin sensitizing potential of isopyrazam, isopyrazam (92.8:7.2 *syn*:*anti*, batch No. SMU6AP001, purity 96.45%) was tested in female CBA/ca/Ola/hsd mice. Animals were exposed to approximately 25 µl of 0%, 10%, 25% or 60% weight per volume (w/v) isopyrazam in dimethyl sulfoxide (DMSO). A positive response was obtained with 60% w/v isopyrazam. Thus, isopyrazam with a high *syn* epimer fraction exhibited skin sensitizing potential in female mice (Betts, 2006).

2.2 Short-term studies of toxicity

Mice

Isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 92.8:7.2 *syn*:*anti*) was administered for 90 days in the diet to groups of 10 male and 10 female 5- to 6-week-old C57BL/10JfCD-1 mice in a range-finding study for a carcinogenicity study. The concentrations in feed were 0, 500, 2500 and 7000 parts per million (ppm) (equal to 0, 76.5, 390.8 and 1382.8 mg/kg bw per day in males and 0, 87.2, 448.9 and 1759.6 mg/kg bw per day in females). Detailed clinical observations, body weights and feed consumption were recorded weekly. In week 12, urine was collected for urinalysis, and a functional observational battery (FOB) was performed. At study termination, haematology, clinical chemistry, organ weights and liver histology from all animals were investigated. Histology in all other organs was performed only in the control and high-dose groups.

Towards the end of the study, body weights in male and female mice were statistically significantly lower by approximately 5–7% at 2500 ppm and by approximately 11% at 7000 ppm. Feed consumption was elevated by approximately 10% in both sexes at 500 and 2500 ppm and by 38% in males and by 54% in females at 7000 ppm. As a result, feed conversion was low at 7000 ppm. At 7000 ppm, females had statistically significantly lower mean haemoglobin concentration, haematocrit and mean cell volume compared with control animals. At 7000 ppm, males had lower red blood cell counts and statistically significantly lower red blood cell distribution width than controls at all dose levels. Platelet counts were statistically significantly increased for all male groups, but without a clear dose–response relationship, and for females at 7000 ppm. In males at 7000 ppm, eosinophils were statistically significantly higher and monocytes were lower than in controls. In females at 7000 ppm, the plasma albumin to globulin ratio was lowered, triglyceride levels were increased by 100% and alanine aminotransferase (ALT) activity was increased by 90%. Body weight–adjusted brain weights were statistically significantly increased by 5% in all dosed males. Body weight–adjusted liver weights were increased dose relatedly in both sexes at all dose levels: by approximately 8% at 500 ppm, 25–32% at 2500 ppm and 47–59% at 7000 ppm. Histological findings were confined to the liver. In all males of all treated groups, hepatocellular hypertrophy was observed, which was slightly more pronounced at 2500 ppm than at 500 ppm and was most pronounced at 7000 ppm. In females, no hepatocellular hypertrophy was observed at 500 ppm, but hepatocellular hypertrophy was evident in 9 of 10 and 10 of 10 animals at 2500 ppm and 7000 ppm, respectively. In one male and one female high-dose animal, necrotic liver nodules were also observed.

The no-observed-adverse-effect level (NOAEL) was 2500 ppm (equal to 390.8 mg/kg bw per day), based on haematological changes at 7000 ppm (equal to 1382.8 mg/kg bw per day) (Pinto, 2008b).

Rats

In a study to compare the toxicity of the *syn* and *anti* conformation isomers of isopyrazam, pure *syn* epimer SYN534969 technical (batch No. SMU6BP001, 99.0% pure), pure *anti* epimer

SYN534968 technical (batch No. SMU6CP001, 95.5% pure) and 1:1 *syn*:*anti* epimer SYN520453 technical (batch No. SMU6CP014, 98.2% pure) were administered for 28 days in the diet to groups of five male and five female 6- to 7-week-old HsdRCCHan:WIST rats. The concentrations in feed were 0, 500, 2000 and 5000 ppm (equal to 0, 45.2, 176.7 and 437.3 mg/kg bw per day in males and 0, 45.3, 187.4 and 401.0 mg/kg bw per day in females) for all three compounds. Clinical observations and body weight were recorded daily, and feed consumption was recorded weekly. At study termination, haematology and clinical chemistry parameters were analysed and organ weights were recorded. Organs of all main study low-dose and high-dose animals as well as livers from the intermediate-group animals were examined histologically. The 7-ethoxyresorufin *O*-deethylase (EROD, cytochrome P450 [CYP] 1A1/2) and 7-pentoxyresorufin *O*-dealkylase (PROD, CYP2B1/2) activities of liver microsomes were analysed.

In males, there were no clinical signs at any dose level with any compound. With pure *syn* epimer, there were no clinical signs in females. With pure *anti* epimer, one female in the 5000 ppm group showed hunched posture on days 6–8, and two females had piloerection on days 10–26. One female in the 2000 ppm group also had a single observation of piloerection on day 12. With 1:1 *syn*:*anti*, there were three females with hunched posture at days 4–8 and four females with piloerection on days 4–26. Feed intake was slightly lower than in controls with pure *syn* in high-dose females (Table 4). With pure *anti* and 1:1 *syn*:*anti*, feed intake was reduced in females at all dose levels and in males at 2000 and 5000 ppm. With pure *anti* and 1:1 *syn*:*anti*, body weights were reduced at 2000 and 5000 ppm in a dose-dependent manner in both sexes and were also slightly reduced in females with both compounds at 500 ppm. With pure *syn*, the body weights of males and females at 5000 ppm were only slightly reduced, mainly during the 1st week of treatment (Table 5). All three compounds had effects on haematological parameters. With pure *syn*, high-dose males had lower platelet and lymphocyte counts, and high-dose females had lower basophilic granulocytes. With pure *anti*, mid- and high-dose females had lower basophilic granulocytes, prothrombin time was increased in females at 5000 ppm and activated partial prothromboplastin time was lower in all treated females. In females at 5000 ppm, haematocrit and haemoglobin levels were increased; in males at this dose level, the red blood cell counts were elevated. With 1:1 *syn*:*anti*, high-dose females had elevated red blood cell counts and slightly lower activated partial prothromboplastin time. All three compounds affected blood clinical chemistry parameters (Table 6). With pure *syn*, cholesterol levels were increased in all dosed females, with a statistically significant increase of 40% at 5000 ppm. In males, cholesterol was increased statistically significantly only at the low and middle doses, but not at the high dose. In high-dose males, alkaline phosphatase (ALP) activity was lowered statistically significantly by 30%. With pure *anti*, cholesterol levels were increased in all dosed females, by 40% statistically significantly at 2000 ppm and by 54% statistically significantly at 5000 ppm. Plasma albumin and total protein were decreased by approximately 10% in females at 2000 and 5000 ppm. In females at 5000 ppm, statistically significant increases in potassium by 21% and in phosphorus by 36% were observed. In males, plasma triglycerides were decreased statistically significantly by 35% at 2000 ppm and by 66% at 5000 ppm. Gamma-glutamyltransferase (GGT) activity was increased in male and female high-dose animals by 24%. The activity of ALT in high-dose females was elevated by 43%; the ALP activity in males was elevated by 33%. With 1:1 *syn*:*anti*, plasma albumin and total protein were decreased by approximately 9% in females at 5000 ppm. Cholesterol levels were increased in all dosed females, by 34% statistically significantly at 2000 ppm and by 63% statistically significantly at 5000 ppm. All dosed females and males at 2000 and 5000 ppm had increased body weight–adjusted liver weights (Table 7). At all dose levels in both sexes for all compounds, the total hepatic P450 content was slightly increased, with no dose dependency (Table 8). Similarly, the EROD activity was elevated in all dosed groups, but not in a compound-, dose- or sex-related manner. PROD activity was increased significantly and dose relatedly, but the increase was not significantly different between sexes and compounds. Hepatocellular hypertrophy was observed in all males in all dose

Table 4. Feed intake in male and female rats exposed to pure syn, pure anti and 1:1 syn:anti isopyrazam

	Feed intake (% of control group)					
	Males			Females		
	500 ppm	2000 ppm	5000 ppm	500 ppm	2000 ppm	5000 ppm
Pure *syn*						
Week 1	120	112	102	100	99	88
Week 2	110	102	104	103	103	104
Week 3	115	103	102	101	98	94
Week 4	112	104	100	97	93	81
Pure *anti*						
Week 1	110	92	66	90	69	55
Week 2	97	91	83	97	87	79
Week 3	101	93	86	93	107	69
Week 4	100	90	78	88	75	63
1:1 *syn*:*anti*						
Week 1	96	88	78	94	83	50
Week 2	99	105	100	96	108	78
Week 3	103	103	96	92	99	69
Week 4	102	103	91	86	98	62

From Milburn (2007e)

Table 5. Terminal body weight in male and female rats exposed to pure syn, pure anti and 1:1 syn:anti isopyrazam

	Body weight (% of control group)					
	Males			Females		
	500 ppm	2000 ppm	5000 ppm	500 ppm	2000 ppm	5000 ppm
Pure *syn*	100	100	95	100	100	95*
Pure *anti*	100	89**	80**	96	83**	82**
1:1 *syn*:*anti*	100	94	86**	97	89**	79**

From Milburn (2007e)
* $P < 0.05$; ** $P < 0.01$

Table 6. Blood clinical chemistry parameter changes in male and female rats exposed to pure syn, pure anti and 1:1 syn:anti isopyrazam

	Parameter changes (% of control group)					
	Males			Females		
	500 ppm	2000 ppm	5000 ppm	500 ppm	2000 ppm	5000 ppm
Pure *syn*						
Cholesterol	+39*	+39*	+6	+25	+13	+40**
Pure *anti*						
Cholesterol	+11	+13	+11	+25	+40**	+54**
Total protein	+2	+2	+4	+4	−11**	−9**
Potassium	+2	+14	+5	+4	+6	+21*

Table 6 (continued)

	Parameter changes (% of control group)					
	Males			Females		
	500 ppm	2000 ppm	5000 ppm	500 ppm	2000 ppm	5000 ppm
Phosphorus	−3	+19	−10	+18	+15	+36*
Triglycerides	−21	−35*	−66**	+42*	+28	−16
GGT	−2	+23*	+24*	−10	−9	+24*
ALT	−10	+12	+16	+29	+2	+43*
1:1 *syn*:*anti*						
Cholesterol	+11	0	+6	+23	+34*	+63**
Total protein	−1	−1	+3	-2	0	−9*

From Milburn (2007e)
ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; * $P < 0.05$; ** $P < 0.01$

Table 7. Body weight–adjusted liver weight changes in male and female rats exposed to pure syn, pure anti and 1:1 syn:anti isopyrazam

	Liver weight changes (% of control group)					
	Males			Females		
	500 ppm	2000 ppm	5000 ppm	500 ppm	2000 ppm	5000 ppm
Pure *syn*	—	+17**	+25**	+22**	+31**	+41**
Pure *anti*	—	+20**	+23**	+21**	+20**	+38**
1:1 *syn*:*anti*	—	+18**	+30**	+15**	+34**	+25**

From Milburn (2007e)
— no effect; * $P < 0.05$; ** $P < 0.01$

Table 8. Hepatic P450 content and PROD and EROD activity changes in male and female rats exposed to pure syn, pure anti and 1:1 syn:anti isopyrazam

	Changes (expressed as ratio to control group values)					
	Males			Females		
	500 ppm	2000 ppm	5000 ppm	500 ppm	2000 ppm	5000 ppm
Pure *syn*						
P450	1.4*	2.1**	1.9**	1.3	1.6*	1.4
PROD	88.7**	133.8**	159.7**	128.6**	215.3**	180.0**
EROD	2.2**	3.1**	2.9**	1.4	2.1**	1.7**
Pure *anti*						
P450	1.1	1.6**	1.4	0.8	0.8	1.1
PROD	35.7**	161.6**	201.7**	42.9**	159.7**	384.5**
EROD	1.6*	2.6**	2.2**	1.2	1.4*	2.0**
1:1 *syn*:*anti*						
P450	1.6**	2.2**	1.7**	1.3	1.3	2.0**
PROD	77.4**	164.3**	283.4**	62.9**	181.2**	263.2**
EROD	2.3**	3.6**	3.7**	1.4	1.7**	2.1**

From Milburn (2007e)
P450, cytochrome P450; EROD, 7-ethoxyresorufin *O*-deethylase; PROD, 7-pentoxyresorufin *O*-dealkylase; * $P < 0.05$; ** $P < 0.01$

groups with all three compounds, becoming increasingly pronounced (minimal/slight/moderate) with increasing dose. The only exception was with animals receiving 500 or 2000 ppm 1:1 *syn*:*anti*, where it was equally pronounced. In all female dose groups with all three compounds, the incidence and extent of hepatocellular hypertrophy increased with dose.

Regarding feed consumption, body weight development and haematological and clinical chemistry parameters, females were more sensitive than males for all three compounds. The severity of the effects was highest with pure *anti* isomer, followed by 1:1 *syn*:*anti* and then by pure *syn* isomer. Liver weight was increased in all dosed female groups and in all male groups at 2000 ppm and above. For liver weight increase, no compound-specific sensitivity was observed. No compound or sex difference was observed for effects on liver histology or on total P450 content or PROD or EROD activity. All compounds mildly elevated total hepatic P450 content and EROD (CYP1A1) activity in both sexes, but markedly increased PROD (CYP2B family) activity. The Meeting considered the liver findings as adaptive effects and not toxicologically relevant.

The NOAEL for pure *syn* was 2000 ppm (equal to 176.7 mg/kg bw per day), based on reduced body weight gain and increased cholesterol levels at 5000 ppm (equal to 437.3 mg/kg bw per day).

The NOAEL for pure *anti* and 1:1 *syn*:*anti* was 500 ppm (equal to 45.2 mg/kg bw per day), based on reduced body weight gain and increased cholesterol levels at 2000 ppm (equal to 176.7 mg/kg bw per day) (Milburn, 2007e).

Isopyrazam (SYN520453 technical, batch No. TE-5854/7, purity not certified, 89:11 *syn*:*anti*) was administered for 28 days in the diet to groups of five male and five female 6- to 7-week-old HsdRCCHan:WIST rats. The concentrations in feed were 0, 100, 500 and 2000 ppm (equal to 0, 9.1, 46.1 and 174.9 mg/kg bw per day in males and 0, 9.6, 48.1 and 191.2 mg/kg bw per day in females). Detailed clinical observations and body weight were recorded twice weekly, and feed consumption was recorded weekly. At week 4, control and high-dose animals were subjected to a FOB, and locomotor activity was monitored within the detailed clinical observation. During week 4, urine from control and high-dose animals was collected for urinalysis. At study termination, haematology, clinical chemistry, organ weights and histology (including livers of low- and mid-dose animals) were investigated in control and high-dose animals. The EROD and PROD activities of liver microsomes were analysed.

There were no treatment-related clinical signs or effects in the FOB. In females at 2000 ppm, there was a slight decrease in feed consumption; concomitantly, these females had slightly lower body weights (maximally 7% compared with controls). In males at 2000 ppm, lymphocyte counts were decreased statistically significantly by 30%, and females had a prolonged activated partial thromboplastin time. At 2000 ppm, creatinine levels (+20%) and creatinine kinase activity (+56%) were increased, triglyceride levels were lowered by 39% in males and urea levels were increased by 18%. High-dose males had statistically significantly higher body weight–adjusted brain weights (+4%) and liver weights (+13%). In high-dose females, body weight–adjusted liver weights were increased statistically significantly by 14%. One male in the 500 ppm group and all males and one female in the 2000 ppm group showed centrilobular hypertrophy. At 2000 ppm, total hepatic P450 content was slightly increased in both sexes and EROD activity was moderately increased in males and females. PROD activity was significantly increased at 500 and 2000 ppm in females and at 2000 ppm in males (for 500 ppm males, no data were generated) (Table 9).

The NOAEL was 500 ppm (equal to 46.1 mg/kg bw per day), based on clinical chemistry and haematological changes at 2000 ppm (equal to 174.9 mg/kg bw per day) (Milburn, 2007a).

Isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 92.8:7.2 *syn*:*anti*) was administered for 28 days in the diet to groups of five male and five female 5- to 6-week-old

Table 9. Hepatic P450 content and PROD and EROD activity changes in male and female rats exposed to isopyrazam

	Changes (expressed as ratio to control group values)			
	Males		Females	
	500 ppm	2000 ppm	500 ppm	2000 ppm
P450	—	3.0**	1.3	1.6*
PROD	—	99.1**	39.9**	74.2**
EROD	—	6.8**	1.5	2.6**

From Milburn (2007a)
P450, cytochrome P450; EROD, 7-ethoxyresorufin *O*-deethylase; PROD, 7-pentoxyresorufin *O*-dealkylase; — not measured; * $P < 0.05$; ** $P < 0.01$

HsdRCCHan:WIST rats. The concentrations in feed were 0, 300, 4000 or 8000 ppm (equal to 0, 29.4, 392.5 and 792.6 mg/kg bw per day in males and 0, 28.1, 390.1 and 720.6 mg/kg bw per day in females). Detailed clinical observations were recorded daily, body weights were measured twice weekly and feed consumption was measured weekly. At study termination, haematology, clinical chemistry, organ weights and liver histology from all animals were investigated.

There were no clinical signs during the study. In the 4000 and 8000 ppm groups, male and female animals had reduced feed consumption and lower body weights compared with control animals. In high-dose males, a slight increase in the prothrombin time was observed. In females, there was a 52% decrease in neutrophil counts at 4000 ppm and a 36% decrease at 8000 ppm, and there was a 34% decrease in eosinophils at 8000 ppm. Several clinical chemistry parameter changes were observed in females at 4000 and 8000 ppm and above (Table 10). In males, triglyceride levels were decreased at all dose levels; at 300 ppm, the effect was not statistically significant. Body weight–adjusted liver weights were increased in both sexes by 22–24% at 4000 ppm and by 28–31% at 8000 ppm. Body weight–adjusted testes weights in 8000 ppm males were increased by 14%. All animals in the 4000 and 8000 ppm groups showed slight to minimal hepatocellular hypertrophy.

The NOAEL was 300 ppm (equal to 28.1 mg/kg bw per day), based on reduced body weight gain and clinical chemistry and haematological changes at 4000 ppm (equal to 390.1 mg/kg bw per day) (Milburn, 2007b).

Isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 92.8:7.2 *syn*:*anti*) was administered for 90 days in the diet to groups of 12 male and 12 female 5- to 6-week-old HsdRCCHan:WIST rats. The concentrations in feed were 0, 300, 1500 and 6000 ppm (equal to 0, 21.3, 106.3 and 463.0 mg/kg bw per day in males and 0, 23.8, 117.8 and 484.4 mg/kg bw per day in females). The eyes of all animals were examined pre-study, and the eyes of the control and high-dose animals were examined during the week prior to termination. Detailed clinical observations, body weights and feed consumption were recorded weekly. In week 12, urine was collected for urinalysis, and a FOB was performed. At study termination, haematology, clinical chemistry, organ weights and liver histology from all animals were investigated. Histology in all other organs was performed only in the control and high-dose groups.

Feed consumption in females was reduced maximally by 12% at 1500 ppm and by 23% at 6000 ppm. The body weights were decreased in these groups by 7% and 19%, respectively. In males at 6000 ppm, feed consumption was reduced by 18% in the 1st week, and body weight was reduced by maximally 8% only in weeks 2 and 3. There were no treatment-related changes in urinalysis and no treatment-related haematological changes. Neutrophil counts were reduced in 300 ppm males by 23%, at 1500 ppm by 22% and at 6000 ppm by 18%. Statistical significance was attained at the low and middle doses. Eosinophil counts were statistically significantly reduced by 29% in high-dose

Table 10. Blood clinical chemistry parameter changes in male and female rats exposed to isopyrazam

	Changes (% of control group)					
	Males			Females		
	300 ppm	4000 ppm	8000 ppm	300 ppm	4000 ppm	8000 ppm
Cholesterol	−18	+2	−15	+5	+33**	+51**
Creatinine	−8	−5	−10*	0	−2	0
Phosphorus	+10	+7	+10	0	+24**	+44**
Triglycerides	−26	−54**	−53**	+9	+16	−4
Urea	−11	−7	−8	−7	+20*	+34**
GGT	+1	+9	+21	+7	+13	+45**

From Milburn (2007b)
GGT, gamma-glutamyltransferase; * $P < 0.05$; ** $P < 0.01$

males. In males, triglycerides and bilirubin were decreased statistically significantly at 1500 and 6000 ppm, and cholesterol was increased dose relatedly, but not statistically significantly (Table 11). At the high dose level, males also showed minimally but statistically significantly elevated levels of sodium, chloride and phosphorus and increased activities of GGT, ALT, aspartate aminotransferase (AST) and creatine kinase. In females, bilirubin was decreased statistically significantly at all dose levels, and GGT activity was increased at 1500 ppm and above. High-dose females also showed minimally but statistically significantly elevated levels of sodium and chloride and increased activities of ALT. In high-dose females, cholesterol was increased statistically significantly. Body weight–adjusted liver weights were increased in females at 300 and 1500 ppm by 14% and at 6000 ppm by 19%. In males, liver weights were increased by 14% at 1500 ppm and by 24% at 6000 ppm. Body weight–adjusted heart weights were statistically significantly decreased in males at 1500 ppm and in both sexes at 6000 ppm. In high-dose females, kidney weights were decreased. In males, thymus weights were decreased statistically significantly by 15% at 300 ppm, by 16% at 1500 ppm and by 23% at 6000 ppm. In other rat feeding studies, thymus weights were not affected. Therefore, the effect here was judged not to be treatment related. At the middle and high dose levels, all male and female animals showed minimal to slight hepatocellular hypertrophy.

The NOAEL was 300 ppm (equal to 21.3 mg/kg bw per day), based on clinical chemistry changes at 1500 ppm (equal to 106.3 mg/kg bw per day) (Milburn, 2007c).

Isopyrazam (SYN520453, batch No. KI-7280/6, purity 99.8%, 97.7:2.3 *syn*:*anti*) was administered for 90 days in the diet to groups of 12 male and 12 female 5- to 6-week-old HsdRCCHan:WIST rats. The concentrations in feed were 0, 250, 1000 and 4000 ppm (equal to 0, 18.9, 74.1 and 314.8 mg/kg bw per day in males and 0, 21.5, 84.8 and 340.7 mg/kg bw per day in females). The eyes of all animals were examined pre-study and prior to termination. Detailed clinical observations, body weights and feed consumption were recorded weekly. In week 12, urine was collected for urinalysis, and a FOB was performed. At study termination, haematology, clinical chemistry, organ weights and liver histology from all animals were investigated. Histology in all other organs was performed only in the control and high-dose groups.

There were no deaths and no compound-related clinical changes, no compound-related effects on the eyes and no compound-related effects on the FOB parameters measured or on motor activity. There were no compound-related effects on haematological or urine clinical chemistry parameters. Males at 4000 ppm had statistically significantly higher platelet counts than controls. Female plasma total protein and plasma calcium were higher in animals at 1000 ppm than in controls. Liver weights

Table 11. Blood clinical chemistry parameter changes in male and female rats exposed to isopyrazam

	Changes (% of control group)					
	Males			Females		
	300 ppm	1500 ppm	6000 ppm	300 ppm	1500 ppm	6000 ppm
Cholesterol	+6	+15	+19	0	+14	+27**
Triglyceride	−4	−28**	−45**	−3	−9	−16
GGT	+4	+4	+22**	+8	+14*	+30**
ALT	+2	+10	+23*	−7	+9	+29*

From Milburn (2007c)
ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; * $P < 0.05$; ** $P < 0.01$

in males and females at 1000 or 4000 ppm were statistically significantly higher than those in controls after adjustment for terminal body weight and showed a dose–response relationship (12% in both sexes at 1000 ppm and 23% and 31% in females and males, respectively, at 4000 ppm). Brain weights in females were higher (3–6%) than those of controls after adjustment for terminal body weight in all dosed animals, statistically significantly at 250 and 4000 ppm. Heart weights in females at 4000 ppm were statistically significantly lower (8%) than controls. Compound-related findings were confined to the liver and consisted of hypertrophy of centrilobular hepatocytes in 10 of 12 males and 8 of 12 females at 1000 ppm and in all animals at 4000 ppm.

The Meeting considered the slightly higher liver weights and hepatocellular hypertrophy in males and females at 1000 ppm as adaptive changes and of no toxicological relevance. The NOAEL was 1000 ppm (equal to 74.1 mg/kg bw per day), based on significantly increased relative liver weights and hepatocellular hypertrophy in males and females at 4000 ppm (equal to 314.8 mg/kg bw per day) (Rattray, 2006b).

In a comparative study, high *syn* isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 92.8:7.2 *syn*:*anti*) or low *syn* isopyrazam (SYN520453, batch No. SMU7DP017, purity 90.8%, 69.7:30.3 *syn*:*anti*) was administered for 90 days in the diet to groups of 10 male and 10 female 6-week-old Crl:WI(Han) rats. The concentrations in feed were 0, 100, 250 and 2000 ppm (equal to 0, 8.3, 20.5 and 161.0 mg/kg bw per day in males and 0, 9.7, 24.1 and 195.0 mg/kg bw per day in females). The eyes of all animals were examined pre-study, and the eyes of the control and high-dose animals were examined during the week prior to termination. Detailed clinical observations, body weights and feed consumption were recorded weekly. In week 12, urine was collected for urinalysis, and a FOB was performed. At study termination, haematology, clinical chemistry, organ weights and liver histology from all animals were investigated. Histology of all other organs was performed only in the control and high-dose groups.

In males treated with low *syn* at 2000 ppm, a statistically significant increase in the incidence of animals with piloerection was observed. At 2000 ppm with both compounds in both sexes, body weight gain was slightly lower than in controls, without any obvious difference between the two compounds. The effect was more pronounced in females. Females in the 2000 ppm groups with both compounds had statistically significantly lower ALP activities. Females treated with high *syn* at 2000 ppm had statistically significantly higher cholesterol levels. In 2000 ppm males, thyroid weights were increased compared with controls, statistical significance being attained only in the high *syn* group. Liver weights in high-dose animals of both sexes were increased with both compounds, and these animals also showed centrilobular hypertrophy and midzonal hepatocyte vacuolation.

There was no obvious difference in toxicity between the two compounds.

The NOAEL for both compounds was 250 ppm (equal to 20.5 mg/kg bw per day), based on body weight effects and effects on the liver at 2000 ppm (equal to 161.0 mg/kg bw per day) (Shearer, 2009).

Dogs

Isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 92.8:7.2 *syn*:*anti*) was administered by gelatine capsule for 90 days to four male and four female 27- to 29-week-old Beagle dogs. The dose levels were 0, 30, 100 and 300 mg/kg bw per day. All dogs were given a full clinical examination by a veterinarian pre-study and prior to termination. Detailed clinical observations and body weights were recorded weekly. Blood samples were taken from all dogs in weeks −2, 4, 8 and 13, and urine samples at weeks −2, 6 and 13. At study termination, organ weights were recorded, and histopathological examinations of all organs were performed.

On day 2 after dosing, two males at 300 mg/kg bw per day showed ataxia and/or inability to stand, slow, jerky forward/backward movements/tremors and absence of some forelimb reflexes. Aggressiveness and excitability with twitching were also observed in one of these animals, and subdued behaviour and increased salivation in another. A third male had reduced activity and slow, side-to-side head movements on day 2 after dosing. One male at 100 mg/kg bw per day showed intermittent, side-to-side head shake/wobble after dosing in week 2 and in week 4. From the start of the study in males and from week 5 in females, body weights were lower by 10–12% in both sexes at 300 mg/kg bw per day. Feed consumption was generally lower in males at 300 mg/kg bw per day throughout the study. Platelet counts in males at 300 mg/kg bw per day were increased compared with controls. Plasma total protein and albumin levels were decreased statistically significantly in both sexes at 300 mg/kg bw per day, in males at study termination and in females from week 4 onwards. Cholesterol levels were dose-relatedly decreased at all dose levels in males and females, although not statistically significantly. ALP activity was increased in both sexes at 100 mg/kg bw per day and above. Urine specific gravity was lower in females at 300 mg/kg bw per day at week 13. Liver weights were increased in males at 100 mg/kg bw per day and in males and females at 300 mg/kg bw per day. In females at all dose levels, ovary, spleen and uterus weights were lowered. In the control group, individual animals showed unusually high weights for these organs, and therefore the decrease in the treated groups seems not to be treatment related.

The NOAEL in this study was 30 mg/kg bw per day, based on behavioural changes and liver weight increases at 100 mg/kg bw per day (Brammer, 2007).

Isopyrazam (SYN520453, batch No. SMU7DP017, purity 90.8%, 69.7:30.3 *syn*:*anti*) was administered by gelatine capsule for 90 days to four male and four female 25- to 27-week-old Beagle dogs. The dose levels were 0, 10, 30 and 250 mg/kg bw per day. All dogs were given a full clinical (including ophthalmoscopy) examination by a veterinarian pre-study and prior to termination. Detailed clinical observations and body weights were recorded weekly. Blood samples were taken from all dogs in weeks −2, 4, 8 and 13, and urine samples in weeks −2, 6 and 13. At study termination, organ weights were recorded, and histopathological examinations of all organs were performed.

In males and females at 250 mg/kg bw per day, salivation was observed during dosing. A low incidence of abnormal behaviours was seen in one dog at this dose level, which comprised sedation, sporadic buckling of the hindlimbs, uncoordinated movements, shaking the head and ptosis. In addition, decreased activity was recorded once in a different male of this dose group. Loose and watery faeces occurred in both males and females at a higher incidence than in controls. Feed consumption in high-dose animals was reduced initially, but recovered throughout the study. One male and one female dog lost weight during the 1st week of treatment.

The NOAEL in this study was 30 mg/kg bw per day, based on clinical observations and initial body weight loss at 250 mg/kg bw per day (Jackson, 2008a).

Isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 92.8:7.2 *syn*:*anti*) was administered by gelatine capsule for 52 weeks to four male and four female 28- to 30-week-old Beagle dogs. The dose levels were 0, 25, 100 and 250 mg/kg bw per day. All dogs were given a full clinical (including ophthalmoscopy) examination by a veterinarian pre-study and prior to termination. Detailed clinical observations (by mistake not done at weeks 1, 4, 9, 10, 12, 15, 17, 24, 26, 30, 36, 37, 41, 46 and 49) and body weights were recorded weekly. Blood samples were taken from all dogs pre-study and in weeks 13, 26 and 52, and urine samples were taken pre-test and in weeks −26 and 52. At study termination, organ weights were recorded, and histopathological examinations of all organs were performed.

There were no treatment-related clinical signs. A decrease in mean feed consumption compared with controls was recorded at 250 mg/kg bw per day during the first 6 weeks of treatment. Feed intake subsequently improved in these animals to levels similar to pre-test, although they remained marginally lower than those of the control animals until approximately week 26. Body weight loss was recorded in three males (7.7%, 3.0% and 7.1% reduction) and two females (6.4% and 4.1% reduction) treated at 250 mg/kg bw per day during week 1 of treatment. There was a statistically significant lower body weight gain in males at 250 mg/kg bw per day and occasional similar statistically significant effects in females. At 100 mg/kg bw per day in males, body weight gain was statistically significantly decreased up to day 85. Plasma albumin in males was statistically significantly, and dose relatedly, lowered at all dose levels in week 13 and thereafter only at 100 and 250 mg/kg bw per day. In females, albumin was lowered at 250 mg/kg bw per day. Bilirubin was lowered in males at 250 mg/kg bw per day in week 13 and at 100 and 250 mg/kg bw per day in week 52. ALP activity was increased at all time points at all dose levels in males, attaining statistical significance at week 13 in the 250 mg/kg bw per day group and only in week 52 (2.2-fold increase) at 25 mg/kg bw per day. ALP activity was increased in females at all dose levels, being statistically significant in week 26 in the 250 mg/kg bw per day group and at week 52 in the 100 mg/kg bw per day group. In high-dose males, ALT and glutamate dehydrogenase activities were increased. In females at 250 mg/kg bw per day, body weight–adjusted heart weights were increased by 19%. In males, liver weights were increased at all dose levels, becoming statistically significant (+42% and +53%, respectively) at 100 and 250 mg/kg bw per day. There were no treatment-related histological changes.

The NOAEL in this study was 25 mg/kg bw per day, based on changes in clinical chemistry and in liver weight at higher dose levels. The occasional modest changes in some clinical chemistry parameters at 25 mg/kg bw per day, in the absence of any corresponding histopathological or other changes, were considered not to be of toxicological significance (Jackson, 2008b).

The overall NOAEL for effects of isopyrazam with a *syn*:*anti* ratio down to 69.7:30.3 in the 3-month and 1-year studies in dogs was 30 mg/kg bw per day.

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 92.8:7.2 *syn*:*anti*) was administered for 80 weeks in the diet to groups of 50 male and 50 female 5- to 6-week-old C57BL/10J$_f$CD-1 mice. The concentrations in feed were 0, 70, 500 and 3500 ppm (equal to 0, 7.8, 56.2 and 432.6 mg/kg bw per day in males and 0, 9.9, 74.9 and 553.6 mg/kg bw per day in females). The eyes of all main study animals were examined pre-study, at week 50 and prior to termination. Detailed clinical observations, body weights and feed consumption were recorded weekly up to week 15 and then every 2 weeks. A FOB was performed on all interim-kill animals. For haematology, blood was taken from all animals at week 53 and at study termination. At study termination, organs were weighed and examined histologically.

A high number of males in the 3500 ppm group (36/50 versus 5/50, 4/50 and 6/50 in the control, 70 ppm and 500 ppm groups, respectively) showed eye discharge at several time points. Body weight gain was reduced in male and female mice at 3500 ppm (Figure 5). In female mice at 70 ppm, the body weights were higher than in controls. In females at 3500 ppm, lymphocyte counts and eosinophil counts were increased by 24% and 43%, respectively. In males at 3500 ppm, body weight–adjusted spleen weights were statistically significantly reduced by 53% and were still statistically significantly reduced by 17% after exclusion of animals with malignant lymphoma and extramedullary haematopoiesis. In this exclusion group, high-dose females also showed statistically significantly reduced spleen weights (−19%). In females at 3500 ppm, kidney weights were statistically significantly reduced by 9%. In both sexes, liver weights were increased statistically significantly by 13% in the 500 ppm female group and by 38% in the 3500 ppm female group. In males at 3500 ppm, liver weights were increased by 24%.

In the liver, the incidence of hepatocellular hypertrophy was increased at 500 ppm and above in females and at 3500 ppm in males. At 3500 ppm, the incidences of epithelial eosinophilic droplets in the nasal cavity of males and of epithelial eosinophilic droplets in the gall bladder of females were elevated (Table 12).

The incidences of benign and malignant tumours were not increased at any dose level.

The NOAEL was 70 ppm (equal to 9.9 mg/kg bw per day), based on periportal hepatocellular hypertrophy in females at 500 ppm (equal to 74.9 mg/kg bw per day) (Pinto, 2008a).

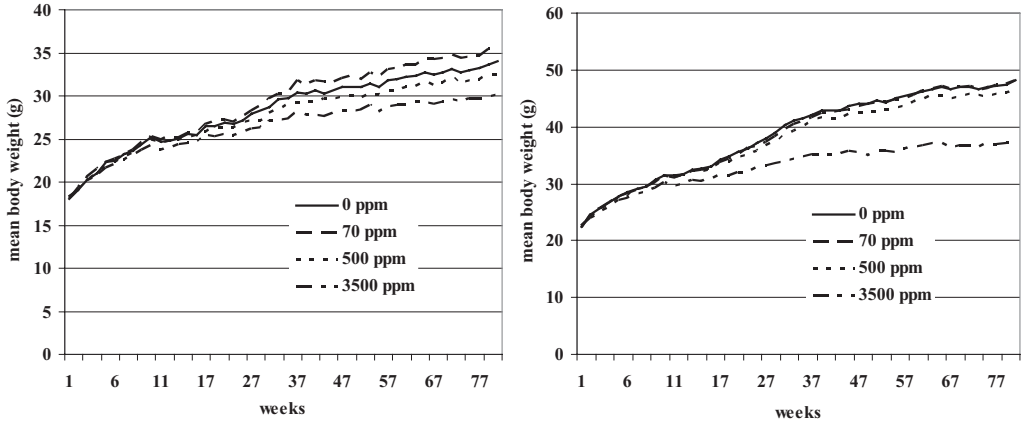
Rats

Isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 92.8:7.2 *syn*:*anti*) was administered for 104 weeks in the diet to groups of 64 male and 64 female 5- to 6-week-old HsdRCCHan:WIST rats. Twelve animals of each sex per dose were investigated at an interim kill at 52 weeks. The concentrations in feed were 0, 100, 500 and 3000 ppm (equal to 0, 5.5, 27.6 and 173.5 mg/kg bw per day in males and 0, 6.9, 34.9 and 232.8 mg/kg bw per day in females). The eyes of all main study animals were examined pre-experimentally, at week 50 and prior to termination. Detailed clinical observations and body weights were recorded weekly up to week 15 and then every 2 weeks. Feed consumption was recorded weekly for the first 13 weeks, then in week 16 and thereafter every 4th week. A FOB of tests and locomotor activity monitoring were performed during week 50 on the interim-kill animals. For haematology, blood was taken from 13 animals of each sex per dose at weeks 14, 27, 53 and 79. From the same animals, urine was collected at weeks 13, 26, 52, 78 and 104. For clinical chemistry, blood was taken from another 13 animals of each sex per dose at weeks 14, 27, 53 and 79. At study termination, organs were weighed and examined histologically.

Seventy-five per cent of males and 67% of females survived until study termination. The finding of "small linear opacity in cornea" was increased in 3000 ppm females (5/36 versus 1/40 in the control group). In males, body weight was lower in the 3000 ppm group by maximally 14% (Figure 6). In females, body weight was statistically significantly lower by maximally 30% at 3000 ppm, by 12% at 500 ppm and by maximally 7% at 100 ppm. Body weight gain was reduced in females by 40% at the high dose, by 16% at the middle dose and by 8% at the low dose. In both sexes, feed consumption at 3000 ppm was reduced. In the FOB, hindlimb grip strength was reduced in high-dose females, and these animals had increased motor activity.

In females, there was a dose-related decrease in haemoglobin, haematocrit, and red blood cell and platelet counts, being statistically significant at 3000 ppm at several time points, predominantly in the second part of the study. In males at 3000 ppm, lymphocyte counts were lowered at all time points, with statistical significance at week 53. In these males, monocyte counts were decreased in the second half of the study, the effect becoming statistically significant at the end of the study. Monocyte counts were also low in high-dose females. At the end of the study, but not at interim kill, the prothrombin time was statistically significantly reduced in males by 3% at 100 ppm, by

Figure 5. Body weights in female (left) and male (right) mice fed isopyrazam in the diet



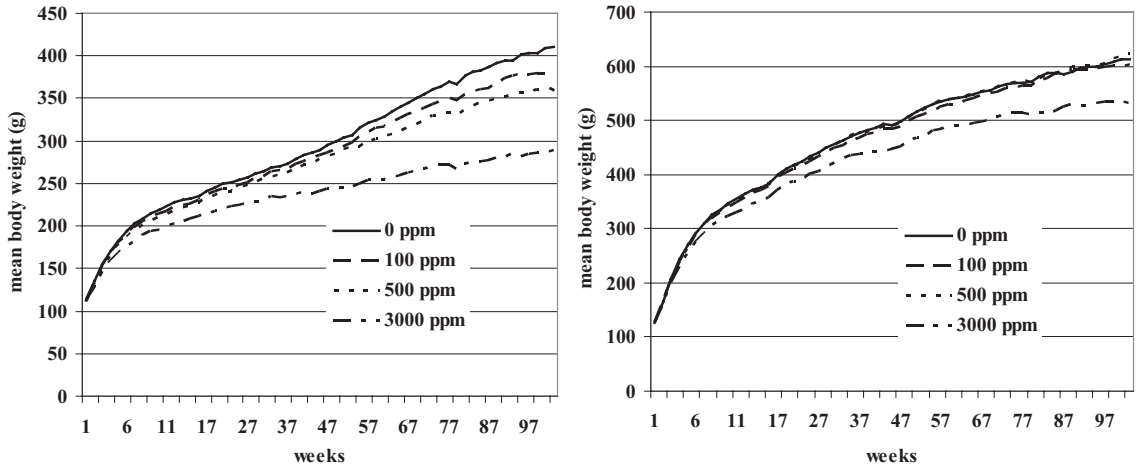
Source: Pinto (2008a)

Table 12. Incidences of non-neoplastic histological changes in male and female mice (terminal kill and preterm deaths combined)

	Severity	Incidence of histological finding							
		Males				Females			
		Dietary concentration (ppm)							
		0	70	500	3500	0	70	500	3500
Hepatocellular hypertrophy									
- midzonal	Minimal	0	0	5	28	—	—	—	—
	Slight	0	0	0	12	—	—	—	—
	Total	0/50	0/50	5/50	40/50**	—	—	—	—
- periportal	Minimal	0	0	0	2	0	0	13	8
	Slight	0	0	0	0	0	0	0	35
	Moderate	0	0	0	0	0	0	0	4
	Total	0/50	0/50	0/50	2/50	0/50	0/50	13/50**	47/50**
Nasal cavity/pharynx									
- epithelial eosinophilic droplets	Minimal	35	28	21	29	19	28	23	32
	Slight	3	6	4	1	2	2	2	4
	Total	38/50	34/50	25/50*	30/50	21/50	30/50	25/50	36/50**
- inflammation/exudate, nasolacrimal duct	Minimal	3	9	11	21	5	2	4	6
	Slight	2	0	1	13	0	1	0	0
	Moderate	1	0	1	4	0	1	0	0
	Total	6/50	9/50	13/50	38/50**	5/50	4/50	4/50	6/50
Gall bladder epithelium, eosinophilic droplets	Minimal	10	3	11	11	9	10	8	18
	Slight	3	3	0	2	1	0	5	3
	Moderate	1	1	0	1	4	0	1	4
	Marked	0	0	0	0	0	1	1	0
	Severe	0	0	1	1	0	0	0	0
	Total	14/48	7/44	12/48	15/49	14/48	11/49	15/50	25/50**

From Pinto (2008a)
— not investigated; * $P < 0.05$; ** $P < 0.01$

Figure 6. Body weights in female (left) and male (right) rats fed isopyrazam in the diet



Source: Milburn (2008)

6% at 500 ppm and by 7% at 3000 ppm. A similar, dose-related effect was observed in females, becoming statistically significant at 3000 ppm. In 500 and 3000 ppm females, the activated partial thromboplastin time was prolonged statistically significantly by 8% at the interim kill and by 4–5% at the end of the study.

Plasma urea was increased (statistically significantly at weeks 53 and 105) by maximally 22% in females at 3000 ppm. In these females, urea was increased at all dose levels, statistically significantly at 100 and 3000 ppm. Plasma creatinine level was elevated statistically significantly at weeks 53 and 79, but not at week 105. Females at 3000 ppm had increased albumin and total protein levels at all time points in the second half of the study, being statistically significant at the end of the study. Cholesterol levels in high-dose females were increased at all time points except at week 105, being statistically significant at weeks 14, 53 and 79. Triglyceride levels in high-dose males were decreased at 500 ppm, statistically significantly at week 27, and at all time points in the 3000 ppm group. In females, triglyceride levels were decreased at all dose levels, statistically significantly at weeks 27, 53 and 79 in the 100 ppm group and at weeks 53, 79 and 105 in the 500 and 3000 ppm groups. In females, bilirubin was dose-relatedly decreased in all dose groups, being statistically significant at week 79 in the 100 ppm group and at most time points in the 500 and 3000 ppm groups. In males and females at 500 and 3000 ppm, ALP activity was decreased statistically significantly at several time points. GGT activity was statistically significantly increased in males at 500 and 3000 ppm in the second half of the study; in females, there was no clear dose–response relationship. ALT activity was increased in high-dose males. AST activity was lowered statistically significantly at week 105 in females at 500 ppm and in the second half of the study at 3000 ppm (Table 13). Sodium and chloride levels were statistically significantly elevated in the 3000 ppm females at most time points. There were no meaningful observations in urinalysis.

At the interim kill at week 52, body weight–adjusted adrenal weights in females in the 3000 ppm group were decreased statistically significantly by 18%. Relative brain weights were increased by 4% in males and decreased by 10% in females. Relative liver weights in both sexes at 3000 ppm were statistically significantly increased by 17% in males and by 14% in females. At terminal kill, relative brain weights in females at 3000 ppm were increased by 4%. Relative liver weights were increased in both sexes, by 5% in males and by 12% in females at 500 ppm and by 17% in males and by 26% in females at 3000 ppm. Absolute and relative adrenal weights in females were lower by 16% at 3000 ppm.

Table 13. Changes in clinical chemistry parameters in rats administered isopyrazam

	Week	% change compared with control					
		Males			Females		
		100 ppm	500 ppm	3000 ppm	100 ppm	500 ppm	3000 ppm
Urea	53	2	−2	2	0	0	9*
	104	0	−4	−16	11*	9	22**
Albumin	53	1	0	−2**	1	−2	1
	104	−1	1	1	−2	−1	3**
Protein	53	2	1	0	0	−1	1
	104	−1	2	0	-2	0	3*
Cholesterol	53	7	15	15	9	8	31*
	104	9	24**	7	−1	−2	0
Triglycerides	53	8	−18	−29*	−20*	−25*	−39**
	104	−9	−14	−37**	1	−25**	−36**
Bilirubin	53	−1	12	−6	−2	−20*	−11
	104	9	−11	−11	−7	−29**	−30**
ALP	53	5	−8	−34**	−3	−24*	−23*
	104	3	−17**	−34**	5	7	−31*
GGT	53	13	18**	25**	1	14	4
	104	11	25**	50**	7	10	11**
ALT	53	−1	2	65*	26	9	−1
	104	31	1	56*	−11	−21*	−7
AST	53	−5	1	41	54	6	−23
	104	6	−10	2	−3	−22*	−21*

From Milburn (2008)
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase;
* $P < 0.05$; ** $P < 0.01$

Histologically, statistically significantly higher incidences of hepatocellular hypertrophy were observed at all dose levels in both sexes at the terminal kill and at 500 ppm in females at the interim kill (Tables 14 and 15). Statistically significantly increased incidences of hepatocellular pigmentation in females were observed at all dose levels at the end of the study and in high-dose females at the interim kill. Males at the interim kill and at study termination were affected statistically significantly only at the high dose level. Incidences of bile duct hyperplasia or fibrosis were increased statistically significantly in males at all dose levels at the study termination. In a contemporary control study conducted in the same laboratory (PR1359), the incidence of minimal bile duct hyperplasia at 24 months was 6 of 52 in males and 8 of 52 in females. The incidence of epithelial mineralization of the pelvis was decreased statistically significantly in high-dose animals. The incidences of foci of eosinophilic hepatocytes were increased statistically significantly at 500 ppm and above in both sexes at study termination.

In females at 3000 ppm, a statistically significant number of hepatocellular adenomas (17%) was observed (Table 16). At 3000 ppm, one hepatocellular carcinoma was found in each sex. Again in the high-dose females, the incidence (23%) of uterine endometrial adenocarcinoma was statistically significantly increased. According to the sponsor, uterine endometrial adenocarcinoma may occur at up to 7.8% in three in-house control groups, and incidences up to 28% are reported in the Registry of Industrial Toxicology Animal-data (RITA) database.

Table 14. Incidences of non-neoplastic histological changes in male and female rats at interim kill

	Severity	Incidence							
		Males				Females			
		Dietary concentration (ppm)							
		0	100	500	3000	0	100	500	3000
No. of animals investigated		*12*	*12*	*12*	*12*	*12*	*12*	*12*	*12*
Hepatocellular hypertrophy	Minimal	0	0	4	5	0	0	9	0
	Slight	0	0	0	7	0	0	0	12
	Total	0	0	4	7**	0	0	9**	12**
Pigmentation hepatocytes/ Kupffer cells	Minimal	0	0	0	5	3	4	4	10
	Slight	0	0	0	1	0	1	0	0
	Total	0	0	0	6*	3	5	4	10*

From Milburn (2008)
* $P < 0.05$; ** $P < 0.01$

Table 15. Incidences of non-neoplastic histological changes in male and female rats (terminal kill and preterm deaths combined)

	Severity	Incidence							
		Males				Females			
		Dietary concentration (ppm)							
		0	100	500	3000	0	100	500	3000
No. of animals investigated		*52*	*52*	*52*	*52*	*52*	*52*	*52*	*52*
Hepatocellular hypertrophy, centrilobular	Minimal	0	12	23	4	0	22	13	1
	Slight	0	0	20	22	0	0	35	13
	Moderate	0	0	2	23	0	0	1	36
	Total	0	12**	43**	49**	0	22**	49**	50**
Pigmentation hepatocytes, centrilobular	Minimal	0	0	1	26	3	34	34	21
	Slight	0	0	0	3	0	0	14	16
	Moderate	0	0	0	3	0	0	1	8
	Marked	0	0	0	0	0	0	0	1
	Total	0	0	1	32**	3	34**	49**	46**
Eosinophilic hepatocytes, focal	Minimal	6	10	18	20	9	14	21	22
	Slight	1	0	5	9	0	1	5	7
	Moderate	0	0	0	3	0	0	0	0
	Total	7	10	23**	32**	9	15	26**	29**
Hepatocellular vacuolation, centrilobular	Minimal	3	7	32	34	1	0	16	3
	Slight	0	0	0	4	0	0	1	0
	Moderate	0	0	0	1	0	0	0	0
	Marked	0	0	0	0	0	0	1	0
	Total	3	7	32**	39**	0	0	18**	3
Bile duct hyperplasia[a]	Minimal	3	10	18	12	15	11	16	20
	Slight	0	1	1	0	0	1	1	1
	Moderate	0	0	0	0	0	0	0	2
	Total	3	11*	19**	12*	15	12	17	23

Table 15 (continued)

	Severity	Incidence							
		Males				Females			
		Dietary concentration (ppm)							
		0	100	500	3000	0	100	500	3000
Bile duct fibrosis	Minimal	1	5	9	12	6	6	11	11
	Slight	0	0	1	0	0	0	1	0
(including hyperplasia)	Total	1 (1)	5 (5)	10 (10)**	12 (10)**	6 (5)	6 (5)	12 (9)	11 (11)
Epithelial mineralization of renal pelvis	Minimal	16	13	14	4	38	41	31	9
	Slight	2	0	0	1	0	0	1	0
	Total	18	13	14	5**	38	41	32	9**
Brown pigmentation in kidney tubules	Minimal	0	1	0	0	18	24	30	39
	Slight	0	0	0	0	0	0	0	3
	Total	0	1	0	0	18	24	30	42**

From Milburn (2008)
* $P < 0.05$; ** $P < 0.01$
[a] Historical control data from 32 studies: males 25% ± 17%, range 0–56%; females 31% ± 18%, range 0–68%.

Table 16. Incidences of neoplastic changes in male and female rats at terminal kill and preterm deaths combined

	Incidence							
	Males				Females			
	Dietary concentration (ppm)							
	0	100	500	3000	0	100	500	3000
No. of animals investigated	*64*	*64*	*64*	*64*	*64*	*64*	*64*	*64*
Hepatocellular adenoma	1	0	0	3	0	1	1	11**
Hepatocellular carcinoma	0	0	0	1	0	0	0	1
Thyroid follicular cell adenoma	1	4	2	7	5	1	3	5
Thyroid follicular cell carcinoma	0	0	5	0	0	1	0	0
Uterine endometrial adenoma	—	—	—	—	1	0	1	0
Uterine endometrial adenocarcinoma	—	—	—	—	1	2	3	15**

From Milburn (2008)
** $P < 0.01$

The NOAEL was 100 ppm (equal to 5.5 mg/kg bw per day), based on reduced body weight gain in females and increased incidences of foci of eosinophilic hepatocytes and clinical chemistry changes in both sexes at 500 ppm (equal to 27.6 mg/kg bw per day) (Milburn, 2008).

2.4 Genotoxicity

Isopyrazam was tested for genotoxicity in a range of guideline-compliant assays in vitro and in vivo. No evidence for genotoxicity was observed in any test. The results of the genotoxicity tests are summarized in Table 17. It is concluded that isopyrazam is unlikely to be genotoxic.

Table 17. Results of genotoxicity studies with isopyrazam

End-point	Test system	Concentration	Lot No.; purity (%)	Result	Reference
Reverse mutation (Ames)	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 *Escherichia coli* WP2 and WP2 uvrA	5.0–5000 µg/plate (±S9)	SMU6AP001; 96.4	Negative	Callander (2006)
	S. typhimurium TA98, TA100, TA1535, TA1537 *E. coli* WP2 and WP2 uvrA	3.0–5000 µg/plate (±S9)	SMU7DP017; 90.8	Negative	Sokolowski (2008c)
Mouse lymphoma TK	L5178Y cell line	0.63–30 µg/ml (±S9)	SMU6AP001; 96.4	Negative	Clay (2006)
Mouse lymphoma TK	L5178Y cell line	1.4–66 µg/ml (±S9)	SMU7DP017; 90.8	Negative	Wollny (2008c)
Chromosomal aberration	Human lymphocytes	10–50 µg/ml (±S9)	SMU6AP001; 96.4	Negative	Fox (2006a)
Chromosomal aberration	Human lymphocytes	3.0–90.5 µg/ml (±S9)	SMU7DP017; 90.8	Negative	Bohnenberger (2008c)
Micronucleated bone marrow cells	Male rat	2000 mg/kg bw	SMU6AP001; 96.4	Negative	Fox (2006c)
Unscheduled DNA synthesis	Primary hepatocytes of male rats	2000 mg/kg bw	SMU6AP001; 96.4	Negative	Fox (2006b)

DNA, deoxyribonucleic acid; S9, 9000 × *g* rat liver supernatant; TK, thymidine kinase

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

Isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 92.8:7.2 *syn*:*anti*) was administered in the diet to groups of 26 male and 26 female 6-week-old HsdRCCHan:WIST rats. The concentrations in feed were 0, 100, 500 and 3000 ppm. The doses achieved are depicted in Table 18. After a 10-week pre-mating phase under exposure, F_0 animals were mated and allowed to rear two F_1 litters. F_{1A} parents were mated after a 10-week pre-mating period and produced a single F_2 generation. Clinical observations, body weight development and feed consumption were recorded regularly (weekly or twice weekly). Reproductive performance was assessed based on successful mating (litter with at least one pup alive), length of gestation and pre-coital interval. F_{1A} pups were separated from the dams at postnatal day (PND) 29 and reared as F_1 parents to produce the F_2 generation. Pups were investigated for clinical conditions, survival, body weight development, anogenital distance, vaginal opening and preputial separation. At termination of F_0 and F_1 parents, organ weights and histology, sperm number, morphology, motility and spermatid resistance to homogenization were recorded. In pups, organs were weighed and examined macroscopically.

There were no significant clinical observations in F_0 or F_1 parental animals. Body weights in F_0 and F_1 animals were lower in females at 500 and 3000 ppm and in males at 3000 ppm throughout pre-mating and gestation. After parturition, body weights in the female 500 ppm group were similar to those of controls.

The number of estrous cycles in the 21-day observation period was statistically significantly higher in F_1 females in the 100 and 500 ppm groups than in the control group, but in the absence of a similar finding in the high-dose group and as the number of cycles in all groups was generally four or five, this is considered to be an incidental finding.

Table 18. Doses achieved in the multigeneration study in rats

	Doses (mg/kg bw per day)					
	Males			Females		
	100 ppm	500 ppm	3000 ppm	100 ppm	500 ppm	3000 ppm
F_0 parents pre-mating	8.3	41.2	250.1	9.3	46.6	276.6
F_1 parents pre-mating	9.5	47.8	288.5	10.2	50.1	301.3
F_0 females during gestation	—	—	—	7.4	37.2	217.4
F_1 females during gestation	—	—	—	8.1	40.6	239.1
F_0 females postpartum[a]	—	—	—	25.2	118.9	699.6
F_1 females postpartum[a]	—	—	—	24.4	129.1	774.0

From Milburn (2007d)

[a] Feed consumption postpartum assumes that the dam consumed all the feed, although the pups present in the cage are known to start to consume diet from around week 2.

F_{1A} and F_{2A} pup body weights were reduced by maximally 14–21% from PND 15 onwards at 3000 ppm, and mean total litter weights were reduced by 21–23% (averaged over PNDs 1–29). At 500 ppm, mean total litter weights were reduced by 7–8% (averaged over PNDs 1–29). In the 100 ppm group, no effect was observed. F_1 males at 3000 ppm showed statistically significantly delayed preputial (2.3 days) separation, and F_1 females at 3000 ppm showed statistically significantly delayed vaginal opening (2 days). Whereas the males showed statistically significantly reduced body weights (−7%), the body weights of females were unchanged.

Liver weights were increased statistically significantly at 3000 ppm in F_0 males and at 500 ppm and above in F_1 males. In females, liver weights were statistically significantly higher at 500 ppm and above in F_0 animals and at 100 ppm and above in F_1 animals. In F_1 and F_2 pups of both sexes, liver weights were statistically significantly increased at 500 ppm and above. Minimal or slight centrilobular or diffuse hepatocellular hypertrophy was dose-relatedly increased in F_0 and F_1 animals at 500 and 3000 ppm. In F_0 males at 3000 ppm, seminal vesicle weights were statistically significantly lower by 10% compared with controls. In F_0 males, thyroid weights were increased statistically significantly at 500 ppm by 14% and at 3000 ppm by 43%. In F_0 and F_1 3000 ppm females, weights of uterus with cervix were decreased statistically significantly by 27–41%. In F_1 females at 500 and 3000 ppm, adrenal weights were higher by 12–14%. Kidney weights in F_1 animals were dose-relatedly increased at all dose levels in both sexes, statistically significantly in females at all dose levels and in males at 3000 ppm. Ovary weights in high-dose F_0 and F_1 females were statistically significantly lower by 13–27%.

The number of implantations and the number of live and dead fetuses combined were both statistically significantly lower in F_0 and F_1 dams at 3000 ppm compared with the controls.

The NOAELs for parental and postnatal developmental toxicity were both 100 ppm (equal to 8.1 mg/kg bw per day), based on decreased body weight and organ weight changes in parental F_0 and F_1 animals (parental toxicity) and on lower mean total litter weights (postnatal developmental toxicity) at 500 ppm (equal to 40.6 mg/kg bw per day). The NOAEL for reproductive performance was 3000 ppm (equal to 239.1 mg/kg bw per day), the highest dose tested (Milburn, 2007d).

(b) Developmental toxicity

Rats

Isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 92.8:7.2 *syn*:*anti*) was administered by oral gavage from gestation day (GD) 5 to GD 21 to groups of 24 female time-mated

11- to 13-week-old HsdRCCHan:WIST rats. The dose levels were 0, 20, 75 and 250 mg/kg bw per day. Detailed clinical observations, body weights and feed consumption were recorded. All rats terminated at day 22 of gestation were given a macroscopic examination, including external observation and an examination of the thoracic and abdominal viscera. The following developmental parameters were examined: number of corpora lutea, gravid uterus weight, number and position of implantations, number of live fetuses, number of early and late intrauterine deaths, weight and sex of fetuses and external, visceral and skeletal abnormalities and variations.

Two animals in the 250 mg/kg bw per day group were killed on days 20 and 21, respectively, as they had marked body weight loss and severe clinical signs of toxicity. One of these animals may have been dosed into the thorax. In the high-dose group, the body weights and feed consumption were statistically significantly lower than in the control groups throughout the study. Pre-implantation losses, post-implantation losses, early intrauterine deaths and consequently the mean number of live fetuses per litter, mean gravid uterus weights and litter weights were affected in a dose-related manner, statistically significantly compared with the concurrent control group (Table 19). However, the historical control data of five developmental toxicity studies in the same year show that the concurrent control values for pre-implantation losses, post-implantation losses and early intrauterine deaths were lower than those for any of the other control groups and that the treated groups showed values similar to the historical control values. Therefore, these observations are regarded as incidental. At 250 mg/kg bw per day, the mean fetal body weight was statistically significantly decreased. At all dose levels, male fetuses were under-represented compared with the concurrent and the historical control groups.

In the 250 mg/kg bw per day group, one fetus with hydroencephalus and microphthalmia was observed, and in another litter, a fetus with internal hydrocephalus only was noted (historical controls for microphthalmia: 0/1354 fetuses; historical controls for internal hydroencephali: 0/1354 fetuses). Whereas incompletely ossified structures in the skull were observed at statistically significantly lower incidences at 75 mg/kg bw per day and above than in controls, non-ossified cervical centra and incomplete xiphoid cartilage were observed at statistically significantly higher incidences. At 250 mg/kg bw per day, incidences of incompletely or non-ossified caudal arches and hind paws were increased statistically significantly.

The NOAEL for maternal toxicity was 75 mg/kg bw per day, based on reduced body weight and clinical signs of toxicity at 250 mg/kg bw per day. The NOAEL for developmental toxicity was 20 mg/kg bw per day, based on non-ossified cervical centra at 75 mg/kg bw per day (Moxon, 2007).

Isopyrazam (SYN520453, batch No. SMU7DP017, purity 90.8%, 69.7:30.3 *syn*:*anti*) was administered by oral gavage from GD 4 to GD 20 to groups of 24 female time-mated 11-week-old RCCHan:WIST rats. The dose levels were 0, 20, 75 and 200 mg/kg bw per day. Detailed clinical observations, body weights and feed consumption were recorded. All rats terminated at day 21 of gestation were examined externally, and organs were investigated macroscopically. The following developmental parameters were examined: number of corpora lutea, gravid uterus weight, number and position of implantations, number of live fetuses, number of early and late intrauterine deaths, weight and sex of fetuses, and external, visceral and skeletal abnormalities and variations.

At 200 mg/kg bw per day, ventral recumbency and sedation were noted in all 24 dams from the 1st day of treatment throughout the 1st week. Thereafter, these effects and ruffled fur were seen occasionally in a few animals. Feed consumption was reduced statistically significantly by 15% from the 1st day of treatment (GD 4) in animals at 75 mg/kg bw per day and above; concomitantly, the body weight gain was reduced statistically significantly by 5% from the 4th day of treatment in the 75 mg/kg bw per day group. At 200 mg/kg bw per day, animals lost weight from the 2nd day of treatment up to GD 10 and then gained body weight to a modest extent. The final body weight in this group was lower by 5% at 75 mg/kg bw per day and by 17% at 200 mg/kg bw per day.

Table 19. Litter data in a developmental toxicity study in rats

	Dose (mg/kg bw per day)				Historical control data[a]
	0	20	75	250	
No. of animals investigated	23	24	23	22	
Mean live fetuses per litter	13.1	12.0	11.6*	11.3**	
Mean gravid uterus weight (g)	84.3	78.8	76.7*	68.5**	
Litter weight (g)	62.0	57.8	55.6*	50.0**	
Mean fetal weight (g)	4.76	4.86	4.84	4.42*	
Proportion of male fetuses	54.9	43.8*	44.8	44.5*	47.1, 49.8, 48.0, 50.5, 50.8
Pre-implantation losses, implants affected (%)	1.9	8.6**	7.7*	8.1**	7.6, 7.3, 13.0, 6.7, 2.7
Pre-implantation losses, litters affected (%)	21.7	62.5**	52.2	59.0*	
Post-implantation losses, implants affected (%)	1.6	4.2	3.8	8.4**	5.8, 3.2, 9.7, 5.0, 4.0
Post-implantation losses, litters affected (%)	21.7	33.3	30.4	59.1*	
Pre- and post-implantation losses combined, implants affected (%)	3.5	12.2	11.3	16.7	
Early intrauterine deaths, implants affected (%)	1	4.2	3.8	7.5**	5.0, 1.1, 7.8, 4.0, 4.0
Early intrauterine deaths, litters affected (%)	13	33.3	30.4	54.5	
Late intrauterine deaths, implants affected (%)	0.6	0	0	0.9	0.7, 2.1, 1.9, 1.1, 0
Late intrauterine deaths, litters affected (%)	8.7	0	0	4.5	

From Moxon (2007)
* $P < 0.05$; ** $P < 0.01$
[a] Means of five developmental toxicity studies in 2006.

No effects on implantation rates, pre-implantation and post-implantation losses or the number of live fetuses were found. At 75 mg/kg bw per day and above, fetal body weights were statistically significantly lower compared with controls. One fetus at 200 mg/kg bw per day was found with diaphragmatic hernia (historical controls: 1/4277 fetuses). Statistically significantly increased incidences of delayed or absent ossification of cervical vertebrae bodies were observed at 200 mg/kg bw per day and of incompletely ossified sternebrae at 75 mg/kg bw per day and above. Additionally, non-ossified structures in forelimbs and hindlimbs were identified.

The NOAELs for maternal and developmental toxicity were both 20 mg/kg bw per day, based on clinical signs and reduced body weight gain in dams (maternal toxicity) and lower fetal body weights (developmental toxicity) at 75 mg/kg bw per day (Possnecker, 2008).

Rabbits

In a dose range–finding developmental toxicity study, isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 92.8:7.2 *syn*:*anti*) was administered by oral gavage from GD 4 to GD 27 to groups of 10 female (> 17 weeks old) mated Himalayan rabbits. The dose levels were 0, 100, 200 and 400 mg/kg bw per day. Clinical observations, body weights and feed consumption were recorded. All rabbits were terminated at GD 28, and gross macroscopic examination of all internal organs, with emphasis on the uterus, uterine contents, position of fetuses in the uterus and number of corpora lutea, was performed. The uteri (and contents) with live fetuses were weighed. Fetuses were weighed individually and examined for gross external and visceral abnormalities, and the cranium was examined for the degree of ossification. No histopathological examinations of the heads were performed.

There were no effects on feed consumption, body weight gain, implantation rates, pre-implantation and post-implantation losses or number of live fetuses. Sex ratios and fetal body weights

were not affected. In fetuses at 400 mg/kg bw per day, a range of cardiovascular changes, including interventricular septal defects, were observed in 7 of 57 fetuses (6/9 litters affected). In controls, no fetuses were affected, and at the low and middle dose levels, two fetuses and one fetus were affected, respectively. Two high-dose fetuses had slightly smaller eyes compared with controls. Two high-dose fetuses and one mid-dose fetus had multiple vertebral, sternebrae and/or rib abnormalities or slightly asymmetrical skull with frontal to parietal bones fused. On a litter basis, statistically significant increases in the incidence of a branched xiphoid cartilage in the cartilaginous sternum were noted at 200 and 400 mg/kg bw per day. However, on a fetal basis, the incidence was not increased.

In this dose range–finding study, the NOAEL for maternal toxicity was 400 mg/kg bw per day, the highest dose tested. The NOAEL for fetal toxicity was 200 mg/kg bw per day, based on the incidence of small eyes and other abnormalities at 400 mg/kg bw per day (Gerspach, 2008a).

In a second dose range–finding developmental toxicity study, isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 92.8:7.2 *syn*:*anti*) was administered by oral gavage from GD 4 to GD 27 to groups of five female (> 17 weeks old) mated Himalayan rabbits. The dose levels were 0, 600, 800 and 1000 mg/kg bw per day. Clinical observations, body weights and feed consumption were recorded. All rabbits were terminated at GD 28, and gross macroscopic examination of all internal organs, with emphasis on the uterus, uterine contents, position of fetuses in the uterus and number of corpora lutea, was performed. The uteri (and contents) with live fetuses were weighed. Fetuses were weighed individually and examined for gross external and visceral abnormalities, and the cranium was examined for the degree of ossification. Additionally, histopathological examinations of the heads were performed.

Animals at 1000 mg/kg bw per day had slightly lower feed consumption, but body weight gain was comparable to that of controls. Parental necropsy and reproductive parameters were not affected. The sex ratio of fetuses was unchanged. At 1000 mg/kg bw per day, male fetuses had slightly, statistically not significantly lower body weights. At all dose levels, eyes smaller than usually observed were found, and a higher incidence of fetuses with malrotated or flexed limbs was observed (Table 20). At 800 mg/kg bw per day and above, changes in the skull were found, and at 1000 mg/kg bw per day, two fetuses with abnormal lung lobulation and one fetus each with an additional liver lobe and absent kidney and ureter were observed.

The NOAEL for maternal toxicity was 1000 mg/kg bw per day, the highest dose tested. Fetotoxicity as small eyes and malrotated or flexed limbs was observed at all dose levels (Gerspach, 2008b).

In a third dose range–finding developmental toxicity study, isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 92.8:7.2 *syn*:*anti*) was administered by oral gavage from GD 7 to GD 28 to groups of 10 female (24–26 weeks old) mated New Zealand White rabbits. The dose levels were 0, 400, 700 and 1000 mg/kg bw per day. Clinical observations, body weights and feed consumption were recorded. All rabbits were terminated at GD 29, blood samples were taken for clinical chemistry and gross macroscopic examination of all internal organs (including microscopy of livers), with emphasis on the uterus, uterine contents, positions of fetuses in the uterus and the number of corpora lutea, was performed. The uteri (and contents) with live fetuses were weighed. Fetuses were weighed individually and examined for gross external, visceral and skeletal abnormalities.

All treated groups had statistically significantly lower body weights (−4%) compared with controls and dose-relatedly lower feed consumption (by 13–33%). In the mid- and high-dose groups, one dam was killed in extremis at each of GD 23 and GD 21, respectively, after having lost appreciable body weight. One low-dose and one mid-dose dam aborted on GD 25. One high-dose dam had significantly elevated GGT activity, and cholangiofibrosis was noted in this dam at termination. In high-

Table 20. Fetal data in the second dose range–finding developmental toxicity study in rabbits

	Incidence of finding								Historical control data[a]
	Dose (mg/kg bw per day)								
	0		600		800		1000		
	F	L	F	L	F	L	F	L	
No. of fetuses/litters examined	*33*	*5*	*27*	*5*	*23*	*5*	*32*	*5*	
Skull									
- frontoparietal suture irregular	0	0	0	0	1	1	2	2	
- bones fused	0	0	0	0	1	1	1	1	8 (1.2%)
- irregular ossification	0	0	0	0	0	0	2	2	7 (1%)
Limbs flexed or malrotated	0	0	1	1	2	1	4	4**	22 (3.2%)
Lung abnormal lobulation	0	0	0	0	0	0	2	1	
Liver additional lobe	0	0	0	0	0	0	1	1	4 (0.6%)
Kidney and ureter absent	0	0	0	0	0	0	1	1	3 (0.4%)
Eye small									
- 50–75% of normal size	0	0	2	1	0	0	5**	3	
- 75–< 100% of normal size	0	0	9**	3	5**	2	10**	3	
- total small eyes	0	0	10**	3	5**	2	14**	3	1 (0.15%)

From Gerspach (2008b)
F, fetus; L, litter; ** $P < 0.01$
[a] Historical control data from five control groups with 682 fetuses in 101 litters.

dose animals, slightly elevated urea nitrogen levels were noted. The relative liver weights of animals in all dosed groups were increased by 33–66%. Histologically, mid- and high-dose animals had hepatocellular hypertrophy, and the cytoplasm of hepatocytes contained vacuoles, most probably lipid containing. At 1000 mg/kg bw per day, early resorptions were statistically significantly increased, and mean fetal weights were lower than control weights. Small eyes were noted in 1 of 95 control fetuses and 5 of 76 fetuses at 1000 mg/kg bw per day (Table 21). Several ocular changes were observed in three of the five high-dose fetuses with small eyes; the changes included haemorrhagic rings around the iris, reddened eyes and dark areas on the eyes. Furthermore, absent gall bladders, extra papillary muscle in the heart and variations of major blood vessels were observed at all dose levels.

No maternal or developmental NOAEL was identified in this study, as reduced body weight in dams and possibly treatment-related absence of gall bladder in fetuses were observed at all dose levels (Sawhney Coder, 2008b).

In a developmental toxicity study, isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 92.8:7.2 *syn*:*anti*) was administered by oral gavage from GD 7 to GD 28 to groups of 25 female 25-week-old mated New Zealand White rabbits. The dose levels were 0, 30, 150 and 500 mg/kg bw per day. Clinical observations, body weights and feed consumption were recorded. All rabbits were terminated at GD 29, and gross macroscopic examination of all internal organs (including histology of livers), with emphasis on the uterus, uterine contents, positions of fetuses in the uterus and the number of corpora lutea, was performed. The uteri (and contents) with live fetuses were weighed. Fetuses were weighed individually and examined for external, visceral and skeletal abnormalities.

One high-dose dam died on GD 24 after having shown signs of intoxication throughout the study. This female had red discoloured lungs and nine late resorptions. Maternal body weight gain was unaffected in all dose groups. At GDs 7–17, feed consumption in the 500 mg/kg bw per day

Table 21. Fetal data in the third dose range–finding developmental toxicity study in rabbits

	Dose (mg/kg bw per day)							
	0		400		700		1000	
	F	L	F	L	F	L	F	L
No. of fetuses/litters examined	*95*	*10*	*62*	*8*	*66*	*8*	*76*	*9*
Major blood vessel variation	1	1	3	2	2	1	6	3
Extra papillary muscle in heart	6	4	15**	4	7	5	13**	8
Gall bladder absent[a]	1	1	5*	3	6*	3	11**	5
Eye small	1	1	0	0	0	0	5	2

From Sawhney Coder (2008b)
F, fetus; L, litter; * $P < 0.05$; ** $P < 0.01$
[a] Historical control data: mean 3.9%; median 3.0%; 75th percentile 5.1%, range 0–12%.

group was slightly lower than in the control group, and this was associated with reduced defecation. Absolute liver weights were increased in the 150 mg/kg bw per day group by 13.1% and in the 500 mg/kg bw per day group by 36.2%. At 150 and 500 mg/kg bw per day, dose-related increases in the incidence and severity of hepatocellular hypertrophy and cytoplasmic vacuolation were noted (Table 22). The vacuoles were free of polyribosomes and probably represented lipid accumulation.

Slightly lower fetal body weights in the 500 mg/kg bw per day group were probably secondary to the slightly higher litter size. One fetus in the 500 mg/kg bw per day group had bilateral microphthalmia. There were no other dose-related changes in the incidences of external, visceral or skeletal malformations or variations.

The NOAEL for maternal toxicity was 150 mg/kg bw per day, based on a statistically significantly increased incidence of centrilobular hepatocellular vacuolation at 500 mg/kg bw per day. The developmental NOAEL was 150 mg/kg bw per day, based on a single observation of bilateral microphthalmia at 500 mg/kg bw per day. It was noted that although the incidence of this defect was within the historical control range, a treatment-related increase in the incidence of this defect had been observed at higher doses in the range-finding studies (Sawhney Coder, 2008c).

2.6 Special studies

(a) Neurotoxicity

Rats

In an acute neurotoxicity study, a single dose of isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 92.8:7.2 *syn*:*anti*) was administered by gavage to groups of 10 male and 10 female 7-week-old HanRCC:WIST rats. The dose levels were 0, 30, 250 and 2000 mg/kg bw. Animals were observed for 15 days. Detailed clinical observations, body weights and feed consumption were recorded weekly. A FOB was performed, and locomotor activity was recorded. At day 16, animals were killed, and the brains were weighed and preserved together with spinal cord and samples from the peripheral nervous system for microscopic examination.

At 1 hour post-dosing, animals had weak appearance and decreased activity in the 250 and 2000 mg/kg bw groups. In these groups, locomotor activity was also reduced 3 hours post-dosing on the 1st day. These effects were dose dependent in incidence and severity. Body weight gain was reduced during the 1st week after treatment in the 250 and 2000 mg/kg bw groups.

Table 22. Liver histology in rabbit dams in a developmental toxicity study

Histological finding	Severity	Incidence of finding			
		Dose (mg/kg bw per day)			
		0	30	150	500
No. of animals investigated		*23*	*24*	*23*	*23*
Hepatocellular hypertrophy	Minimal	0	[a]	4	12
	Mild	0	[a]	0	2
	Total	0	[a]	4*	14**
Centrilobular hepatocellular vacuolation	Minimal	0	[b]	3	8
	Mild	0	[b]	0	2
	Moderate	0	[b]	0	3
	Total	0	[b]	3	13**
Midzonal hepatocellular vacuolation	Minimal	4	[b]	2	5
	Mild	2	[b]	3	0
	Moderate	0	[b]	0	1
	Total	6	[b]	5	6

From Sawhney Coder (2008c)

* $P < 0.05$; ** $P < 0.01$

[a] Histological evaluation indicated that hepatocyte hypertrophy alteration in four rabbits in the 30 mg/kg bw per day group was minor and less than the minimal grade in the 150 and 500 mg/kg bw per day groups.

[b] No data given.

The NOAEL for systemic effects was 30 mg/kg bw, based on clinical signs of toxicity at 250 mg/kg bw. The NOAEL for acute neurotoxicity was 2000 mg/kg bw, the highest dose tested (Sommer, 2009b).

In a subchronic neurotoxicity study, isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 92.8:7.2 *syn*:*anti*) was administered for 90 days in the diet to groups of 12 male and 12 female 7-week-old HanRCC:WIST rats. The concentrations in feed were 0, 300, 1500 and 6000 ppm. The eyes of all animals were examined pre-study, and the eyes of the control and high-dose groups were examined during the week prior to termination. Detailed clinical observations and body weights were recorded weekly, and feed consumption was recorded twice weekly. A FOB was performed, and locomotor activity was recorded. At week 13, animals were killed, and the brains were weighed and preserved together with spinal cord and samples from the peripheral nervous system for microscopic examination.

Feed consumption was reduced in male and female rats at 6000 ppm, in males by 4% in the 1st week only and in females by 12% during the first half of the study. Concomitantly, body weight gain was reduced in males during the first weeks and in females by 10% throughout the whole study. No other treatment-related effects were observed.

The NOAEL for systemic toxicity was 1500 ppm (equal to 98.01 mg/kg bw per day), based on decreased body weight gain in females at 6000 ppm (equal to 382.26 mg/kg bw per day). The NOAEL for subchronic neurotoxicity was 6000 ppm (equal to 382.26 mg/kg bw per day), the highest dose tested (Sommer, 2009a).

(b) Induction of enzyme activity, DNA synthesis and estrogen receptor alpha binding

Isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 93:7 *syn*:*anti*) was tested for its ability to induce CYP2B (PROD) and CYP2B/3A (benzyloxyresorufin *O*-debenzylase [BROD])

activity and cell proliferation (measured as the change in DNA synthesis [S-phase of the cell cycle]) in isolated primary hepatocytes of female Han Wistar rats. Phenobarbital sodium salt was used as positive control. Hepatocytes were exposed to isopyrazam at 1, 3, 10, 30, 65 and 100 µmol/l and phenobarbital sodium salt at 10, 100 and 1000 µmol/l or vehicle (0.5% DMSO) for 96 hours. Cytotoxicity was assayed by measuring the change in cellular adenosine-5′-triphosphate (ATP) levels.

Both phenobarbital sodium salt and isopyrazam induced PROD and BROD activities and DNA synthesis. Comparing the potencies using the per cent changes at the lowest doses producing a statistically significant effect gives a higher potency of isopyrazam compared with phenobarbital sodium salt, for PROD 17-fold, for BROD 22-fold and for DNA synthesis 12-fold (Table 23) (Elcombe, 2011d).

Isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 93:7 *syn*:*anti*) was tested for its ability to induce CYP2B (PROD) and CYP2B/3A (BROD) activities and cell proliferation (measured as the change in DNA synthesis [S-phase of the cell cycle]) in isolated primary human female hepatocytes. Phenobarbital sodium salt was used as positive control. Hepatocytes were exposed to isopyrazam at 1, 3, 10, 30, 65 and 100 µmol/l and phenobarbital sodium salt at 10, 100 and 1000 µmol/l or vehicle (0.5% DMSO) for 96 hours. Cytotoxicity was assayed by measuring the change in cellular ATP levels.

Phenobarbital sodium salt and isopyrazam did not induce DNA synthesis statistically significantly at any concentration level. Phenobarbital sodium salt statistically significantly induced PROD and BROD activities at 1000 µmol/l. Isopyrazam did not statistically significantly induce PROD activity even at the highest concentration but was a potent inducer of BROD activity at all dose levels tested (Table 24). Comparing the potencies using the per cent changes at the lowest doses producing a statistically significant effect gives a 470-fold higher potency of isopyrazam compared with phenobarbital sodium salt for BROD activity (Elcombe, 2011c).

Isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 93:7 *syn*:*anti*) was tested for its ability to induce activities of CYP1A (EROD), CYP2B (PROxD), CYP2B/3A (BROD), CYP3A (testosterone 6β-hydroxylase) and CYP4A (lauric acid 12-hydroxylase) and cell proliferation (measured as the change in DNA synthesis [S-phase of the cell cycle]) in 5- to 6-week-old female Han Wistar rats. Additionally, detailed clinical observations were recorded weekly, and body weight and feed consumption were recorded daily. ALT and AST activities were measured, and livers were weighed and investigated microscopically. At feed concentrations of 0, 500 or 3000 ppm each, 10 animals were fed for 3, 7 or 14 days.

At several time points, animals in the 500 and 3000 ppm groups showed lower feed intake. In the 3000 ppm group, the body weight gain was initially low, but recovered thereafter. No changes in ALT and AST activities were observed. Relative liver weights were increased from day 4 onwards at 500 and 3000 ppm by approximately 10% and 20%, respectively. At 3000 ppm, 9 of 10 animals showed histologically increased mitosis in the liver at day 4 in the 3000 ppm group, and all animals had minimal to mild hepatocellular hypertrophy at days 8 and 15. The liver S-phase labelling index was elevated 1.5-fold at 500 ppm at day 3 (statistically not significant) and about 2.1-fold at 3000 ppm. At days 8 and 15, S-phase labelling indices were all similar. At both dose levels and at all time points, total CYP content and CYP1A and CYP2B activities were statistically significantly elevated. For CYP1A and CYP2B activities, there was no significant difference in induction potency between the two dose levels. For CYP3A, there was a dose-related decrease in enzyme activity at all three time points (Table 25). There was a slight and statistically significant decrease (17–30%) in peroxisomal palmitoyl coenzyme A metabolism in the 300 ppm group at all time points. The effect was statistically significant at day 3 and day 7 (Murchison, 2010).

Table 23. Change in enzyme activity and DNA synthesis in cultured primary female rat hepatocytes

Treatment	Control	Phenobarbital sodium salt			Isopyrazam				
	Concentration (µmol/l)								
	0	10	100	1000	1	3	10	30	65
ATP[a]	100.0 ± 3.2	101.7 ± 1.7	104.9 ± 5.0	113.1 ± 3.7**	105.6 ± 3.0*	106.6 ± 4.1*	106.4 ± 3.5**	96.7 ± 4.7	2.0 ± 0.1**
DNA synthesis[b]	100.0 ± 11.1	164.9 ± 19.3**	215.9 ± 16.1**	242.7 ± 7.2**	192.2 ± 11.7**	257.4 ± 20.8**	226.4 ± 22.4**	218.4 ± 43.7**	194.5 ± 26.3[c]
PROD[d]	100.0 ± 153.8	109.8 ± 45.8	395.5 ± 73.7*	584.3 ± 67.5**	147.0 ± 96.9	289.6 ± 97.1	664.3 ± 190.8*	408.0 ± 19.8*	286.0 ± 74.7[c]
BROD[d]	100.0 ± 13.6	110.4 ± 19.4	202.8 ± 16.2**	261.6 ± 49.3**	135.2 ± 67.8	136.9 ± 14.0*	221.4 ± 28.6**	274.2 ± 56.9**	111.8 ± 16.4[c]

From Elcombe (2011d)

ATP, adenosine-5′-triphosphate; BROD, benzyloxyresorufin *O*-debenzylase; DNA, deoxyribonucleic acid; PROD, 7-pentoxyresorufin *O*-dealkylase; * $P < 0.05$; ** $P < 0.01$

[a] $n = 6$ per group.

[b] $n = 5$ per group.

[c] No statistical analyses performed (excessive cytotoxicity).

[d] $n = 3$ per group.

Table 24. Change in enzyme activity compared with control and DNA synthesis in cultured primary female human hepatocytes

Treatment	Control	Phenobarbital sodium salt			Isopyrazam			
	Concentration (µmol/l)							
	0	10	100	1000	1	3	10	30
ATP[a]	100.0 ± 7.2	108.8 ± 11.5	106.0 ± 9.2	95.5 ± 10.4	101.1 ± 8.4	98.1 ± 5.8	89.1 ± 5.3*	64.6 ± 7.7**
DNA synthesis[b]	100.0 ± 38.3	122.7 ± 10.4	121.6 ± 40.3	161.7 ± 51.7	85.9 ± 15.2	102.9 ± 37.1	110.8 ± 21.8	117.3 ± 22.9
PROD[c]	100.0 ± 24.2	73.8 ± 10.0	70.5 ± 20.9	183.0 ± 14.1**	92.5 ± 36.6	58.8 ± 30.3	85.9 ± 33.7	167.9 ± 60.5
BROD[c]	100.0 ± 27.7	124.3 ± 12.9	193.5 ± 87.9	632.4 ± 32.4**	294.9 ± 15.5**	383.9 ± 94.3**	420.8 ± 46.0**	490.3 ± 96.0**

From Elcombe (2011c)

ATP, adenosine-5′-triphosphate; BROD, benzyloxyresorufin *O*-debenzylase; DNA, deoxyribonucleic acid; PROD, 7-pentoxyresorufin *O*-dealkylase; * $P < 0.05$; ** $P < 0.01$

[a] $n = 6$ per group.

[b] $n = 5$ per group.

[c] $n = 3$ per group.

Table 25. Change in enzyme activity relative to control in liver microsomes

	% change relative to control											
	CYP1A			CYP2B			CYP3A			CYP4A		
	Day											
	3	8	15	3	8	15	3	8	15	3	8	15
500 ppm	328*	247*	242*	30 303*	11 048*	20 912*	109	100	84	101	113	95
3000 ppm	213*	255*	269*	34 640*	16 195*	33 953*	[b]	74	59*	78*	104	111
3-MC	7750	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne
PB[a]	ne	ne	ne	23 416	ne	ne	ne	ne	ne	ne	ne	ne
DEX[a]	ne	ne	ne	ne	ne	ne	311	ne	ne	ne	ne	ne
CF[a]	ne	ne	ne	ne	ne	ne	ne	ne	ne	574	ne	ne

From Murchison (2010)

CF, clofibrate; DEX, dexamethasone; 3-MC, 3-methylcholanthrene; ne, not evaluated; PB, phenobarbital; * $P < 0.05$

[a] Commercially available hepatic microsomes from 3-MC-, PB-, DEX- and CF-treated Sprague-Dawley rats, used as positive controls.

[b] Below limit of quantification.

Isopyrazam (SYN520453, batch No. SMU6AP001, purity 96.4%, 93:7 *syn*:*anti*) was tested for its ability to act as an agonist of human estrogen receptor alpha (hERα) using the hERα-HeLa-9903 cell line and luciferase as reporter gene. Cell viability was monitored by propidium iodide uptake, and precipitation was determined by a light-scattering procedure using nephelometry. The positive control was 17β-estradiol (10^{-9} mol/l).

Isopyrazam concentrations greater than 10^{-5} mol/l were found to be cytotoxic and induce precipitation. Therefore, isopyrazam concentrations of 10^{-5} mol/l and below were tested for transcriptional activation of hERα. No increase in luciferase activity was observed in this range of isopyrazam concentrations (Toole, 2011).

(c) Rat uterotrophic assay

Isopyrazam (batch No. SMU6AP001, purity 96.4%, 93:7 *syn*:*anti*) was administered for 3 consecutive days at a dose level of 300 mg/kg bw per day by gavage to groups of six 10-week-old ovariectomized (at week 6) female Crl:WI(Han) rats. As a positive control, 17β-ethinylestradiol at a dose of 0.03 mg/kg bw per day was used; as a negative control, dibutylphthalate at a dose of 1000 mg/kg bw per day was used. All animals were observed twice daily for mortality and moribundity. Clinical observations, body weights and feed consumption were recorded daily throughout the study. Animals were terminated on study day 3 (the day following the last dose administration), and a gross examination of the uterus was conducted; uterine weights (wet and blotted) were recorded.

Whereas 17β-ethinylestradiol induced a statistically significant increase in uterine weights by approximately 30%, no effect on uterine weights was observed for isopyrazam or dibutylphthalate (Kuhl, 2011b).

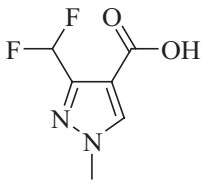
(d) Studies with metabolites CSCD465008 and CSCD459488

CSCD465008, a soil and plant metabolite, and CSCD459488, a soil, plant and aquatic metabolite and also identified in rat in vitro metabolism (White, 2008), were investigated in acute and short-term toxicity studies and genotoxicity studies. CSCD459488 was also investigated in a developmental toxicity study in rabbits. Their structures are illustrated in Figure 7.

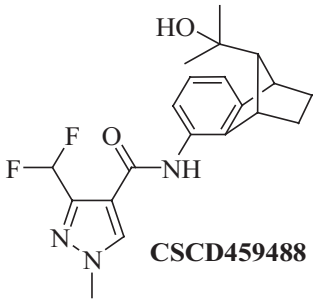
CSCD465008, a soil and plant metabolite of isopyrazam

Acute toxicity. The acute oral toxicity of CSCD465008 (batch No. MES-103/1, purity 94.0 ± 2%) at 2000 mg/kg bw was tested in five female HanRCC Wistar rats. No mortalities were seen, but ruffled

Figure 7. Structures of metabolites CSCD465008 and CSCD459488



CSCD465008



CSCD459488

fur, slight sedation and hunched posture were observed within the first few hours after treatment. The LD_{50} of CSCD465008 in female rats was greater than 2000 mg/kg bw (Simon, 2008b).

Short-term studies of toxicity. CSCD465008 (batch No. MES-103/1, purity 94.0%) was administered for 28 days in the diet to groups of five male and five female 7-week-old Crl:WI(Han) rats. The concentrations in feed were 0, 2000, 6000 and 12 000 ppm (equal to 0, 175, 497 and 1018 mg/kg bw per day in males and 0, 176, 525 and 1107 mg/kg bw per day in females). Clinical observations were recorded daily, and body weights and feed consumption were recorded weekly. At week 3, all animals were subjected to a FOB, and locomotor activity was monitored within the detailed clinical observation. In week 3, all animals underwent an ophthalmic examination. At study termination, haematology, urinalysis and clinical chemistry were analysed. Organs of all animals were examined macroscopically and weighed. A range of tissues from control and high-dose animals was examined histologically. Livers and duodenum were investigated microscopically, and the EROD (CYP1A1/2) and PROD (CYP2B1/2) activities of liver microsomes were analysed. Hepatic microsomes from β-naphthoflavone-treated (100 mg/kg bw for 4 days) and phenobarbital-treated (80 mg/kg bw for 4 days) male and female Sprague-Dawley rats were used as positive controls.

None of the investigated parameters showed any treatment-related changes, and no induction of CYP1A or CYP2B activity was noted (Table 26). The NOAEL was 12 000 ppm (equal to 1018 mg/kg bw per day), the highest dose tested (Walraven, 2008).

Genotoxicity in vitro. CSCD465008 (batch No. MES-103/1, purity 94 ± 2%) was tested for genotoxicity in a range of guideline-compliant assays in vitro. No evidence for genotoxicity was observed in any test. The results are summarized in Table 27.

CSCD459488, a soil, plant and aquatic metabolite of isopyrazam

Acute toxicity. The acute oral toxicity of CSCD459488 (batch No. MES-111/3, purity 99%) at a dose of 2000 mg/kg bw was tested in five female HanRCC:WIST rats. No signs of toxicity were observed. The LD_{50} of CSCD465008 in female rats was greater than 2000 mg/kg bw (Simon, 2008a).

Short-term studies of toxicity. CSCD459488 (batch No. MES-111/3, purity 99%) was administered for 28 days in the diet to groups of five male and five female 7-week-old Crl:WI(Han) rats. The concentrations in feed were 0, 300, 4000 and 10 000 ppm (equal to 0, 27, 370 and 927 mg/kg bw per day in males and 0, 29, 388 and 906 mg/kg bw per day in females). Detailed clinical observations were recorded weekly, and body weights and feed consumption were recorded twice weekly. At week 3, all animals were subjected to a FOB, and locomotor activity was monitored within the detailed clinical observation. In week 3, all animals underwent an ophthalmic examination. At study termination, haematology, urinalysis and clinical chemistry were analysed. Organs of all animals were examined macroscopically and weighed. Organs from the control and high-dose groups and livers from all groups were investigated microscopically, and EROD (CYP1A1/2) and PROD (CYP2B1/2) activities of liver microsomes were analysed. Hepatic microsomes from 3-methylcholanthrene- and phenobarbital-treated

Table 26. CYP1A and CYP2B activities in hepatic liver microsomes of male and female rats treated with CSCD465008

Dietary concentration (ppm)	Activity relative to control (fold change)			
	CYP1A		CYP2B	
	Male	Female	Male	Female
2000	0.9	0.9	1.1	1.0
6000	0.7	0.8	0.9	0.9
12 000	0.7	0.9	1.2	1.0
Phenobarbital	2.8	2.8	23.1	53.8
β-Naphthoflavone	11.9	15.9	1.7	4.9

From Walraven (2008)

Table 27. Results of genotoxicity studies with CSCD465008

End-point	Test system	Concentration	Lot No.; purity (%)	Result	Reference
Reverse mutation (Ames)	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537; *Escherichia coli* WP2, WP2 uvrA	3–5000 µg/plate	MES-103/1; 94	Negative	Sokolowski (2008b)
Mouse lymphoma TK	L5178Y cell line	110–1760 µg/ml	MES-103/1; 94	Negative	Wollny (2008b)
Chromosomal aberration	Human lymphocytes	574.7–1760 µg/ml	MES-103/1; 94	Negative	Bohnenberger (2008b)

TK, thymidine kinase

male and female Sprague-Dawley rats were used as positive controls; the dose levels and duration of treatment were not specified.

In males and females, the body weight–adjusted liver weights were increased by 4–6% at 300 ppm and statistically significantly by 25–33% and by 33–36% at 4000 ppm and 10 000 ppm, respectively. Centrilobular hepatocyte hypertrophy was observed in both sexes at 4000 and 10 000 ppm, but was more pronounced in males. In males at 4000 and 10 000 ppm, minimal follicular cell hypertrophy of the thyroid was noted. In males, total microsomal CYP content was approximately doubled in the 4000 and 10 000 ppm groups and only slightly elevated in females. CYP1A and CYP2B activities were statistically significantly (except males for CYP2B at 300 ppm) increased at all dose levels (Table 28). For CYP1A, the induction at 4000 ppm and above was more pronounced in males; for CYP2B, the induction was more pronounced in females at all dose levels.

The NOAEL was 300 ppm (equal to 27 mg/kg bw per day), based on liver weight changes at 4000 ppm (equal to 370 mg/kg bw per day) (Robertson, 2009).

Developmental toxicity. In a developmental toxicity study, CSCD459488 (batch No. MES 111/3, purity 99%) was administered by oral gavage from GD 7 to GD 28 to groups of 10 female mated 24-week-old New Zealand White rabbits. The dose levels were 0, 150, 500 and 1000 mg/kg bw per day. Clinical observations, body weights and feed consumption were recorded. All rabbits were terminated at GD 29, blood samples were taken for possible analysis of CSCD459488 and macroscopic examination of the uterus, uterine contents, positions of fetuses in the uterus and the number of corpora lutea was performed. Livers were weighed and samples retained for possible microscopic examination. The uteri (and contents) with live fetuses were weighed. Fetuses were weighed individually and examined for gross external, visceral and skeletal abnormalities.

Table 28. CYP1A and CYP2B activities in liver microsomes of male and female rats treated with CSCD459488

Dietary concentration (ppm)	Activity relative to control (fold change)			
	CYP1A		CYP2B	
	Male	Female	Male	Female
300	1.4***	1.9*	2.7	54.0***
4000	3.7**	1.8*	100.2***	117.4*
10 000	4.2***	2.3*	124.6*	78.7***
Phenobarbital[a]	—	—	119.7	—
3-Methylcholanthrene[a]	7.6	—	—	—

From Robertson (2009)
— not tested; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$
[a] No treatment details provided.

The body weight development of dams was not affected by treatment. At all dose levels, maternal liver weights were increased dose relatedly, by 17.5%, 31.8% and 55.1% at 150, 500 and 1000 mg/kg bw per day, respectively. In the high-dose group, late resorptions per litter (5.6%) were statistically significantly increased, primarily due to three resorptions in one female compared with controls (0%). One high-dose fetus had a narrow pectoral region, macroglossia and enlarged lungs and larynx. This very low incidence is not considered to be treatment related in the absence of any other malformation.

The maternal NOAEL was 150 mg/kg bw per day, based on significant liver weight increases greater than 20% at higher doses. The NOAEL for developmental toxicity was 1000 mg/kg bw per day, the highest dose tested (Sawhney Coder, 2008a).

Genotoxicity in vitro. CSCD459488 (batch No. TE-6472/4 or batch No. MES 111/3, purity 99%) was tested for genotoxicity in a range of guideline-compliant assays in vitro. No evidence for genotoxicity was observed in any test. The results are summarized in Table 29.

Induction of enzymes and DNA synthesis. CSCD459488 (SYN545364, batch No. MES 111/4, purity 96%) was tested for its ability to induce CYP2B (PROD) and CYP2B/3A (BROD) activities and cell proliferation (measured as the change in DNA synthesis [S-phase of the cell cycle]) in isolated primary hepatocytes of female Han Wistar rats. Phenobarbital sodium salt was used as positive control. Hepatocytes were exposed to CSCD459488 at 1, 3, 10, 30, 100 and 500 µmol/l and phenobarbital sodium salt at 10, 100 and 1000 µmol/l or vehicle (0.5% DMSO) for 96 hours. Cytotoxicity was assayed by measuring the change in cellular ATP levels.

Both phenobarbital sodium salt and CSCD459488 induced PROD and BROD activities and DNA synthesis (Table 30). Comparing the potencies using the per cent changes at the lowest doses producing a statistically significant effect gives a higher potency of CSCD459488 compared with phenobarbital sodium salt, for PROD 52-fold, for BROD 86-fold and for DNA synthesis 10-fold (Elcombe, 2011b).

CSCD459488 (SYN545364, batch No. MES 111/4, purity 96%) was tested for its ability to induce CYP2B (PROD) and CYP2B/3A (BROD) activities and cell proliferation (measured as the change in DNA synthesis [S-phase of the cell cycle]) in isolated primary female human hepatocytes. Phenobarbital sodium salt was used as positive control. Hepatocytes were exposed to CSCD459488 at 1, 3, 10, 30, 65 and 100 µmol/l and phenobarbital sodium salt at 10, 100 and 1000 µmol/l or vehicle (0.5% DMSO) for 96 hours. Cytotoxicity was assayed by measuring the change in cellular ATP levels.

Phenobarbital sodium salt and CSCD459488 did not induce DNA synthesis statistically significantly at any concentration level. Phenobarbital sodium salt induced PROD and BROD activities at

Table 29. Results of genotoxicity studies with CSCD459488

End-point	Test system	Concentration	Lot No.; purity (%)	Result	Reference
Reverse mutation (Ames)	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537; *Escherichia coli* WP2, WP2 uvrA	3–5000 µg/plate	TE-6472/4; 99	Negative	Sokolowski (2008a)
Mouse lymphoma TK	L5178Y cell line	25–800 µg/ml	MES 111/3	Negative	Wollny (2008a)
Chromosomal aberration	Human lymphocytes	31.8–522.4 µg/ml	MES 111/3	Negative	Bohnenberger (2008a)

TK, thymidine kinase

10 and 100 µmol/l, respectively, statistically significantly and CSCD459488 at 1 and 3 µmol/l (Table 31). Comparing the potencies using the per cent changes at the lowest doses producing a statistically significant effect gives a 9-fold higher potency of CSCD459488 compared with phenobarbital sodium salt for PROD activity and a 30-fold higher potency for BROD activity (Elcombe, 2011a).

CSCD459488 (batch No. MES111/4, purity 96%) was administered for 3 consecutive days at a dose level of 300 or 1000 mg/kg bw per day by gavage to groups of six 9-week-old ovariectomized (at week 6) female Crl:WI(Han) rats. As a positive control, 17β-ethinylestradiol at a dose of 0.03 mg/kg bw per day was used, and as a negative control, dibutylphthalate at a dose of 1000 mg/kg bw per day was used. All animals were observed twice daily for mortality and moribundity. Clinical observations, body weights and feed consumption were recorded daily throughout the study. Animals were terminated on study day 3 (the day following the last dose administration), and a gross examination of the uterus was conducted; uterine weights (wet and blotted) were recorded.

Whereas 17β-ethinylestradiol induced a statistically significant increase in uterine weights by approximately 300% (17β-ethinylestradiol was used at a dose 10-fold higher than in the uterotrophic assay with isopyrazam), no effect on uterine weights was observed for CSCD459488 or dibutylphthalate (Kuhl, 2011a).

3. Observations in humans

No reports were submitted.

Comments

Biochemical aspects

Isopyrazam administered to rats at 1 or 75 mg/kg bw was rapidly absorbed, with peak levels of radiolabel occurring in plasma within 3–6 hours post-dosing. Approximately 70% of the dose was absorbed at 1 and 75 mg/kg bw. The terminal half-life of the labelled material was 5–9 hours. No saturation of absorption was observed, but the area under the curve increased disproportionately with dose, indicating saturation of elimination at higher doses. In the low-dose group, 91–97% of the absorbed dose was excreted within 48 hours after administration. Highest residues were identified in the liver, kidney, thyroid and adrenals. The major route of excretion was by bile, accounting for approximately 65–90% of the absorbed dose, with the rest being excreted in urine within 48 hours. After repeated dosing, no accumulation of radioactivity in rats was observed. There were no significant differences between the toxicokinetic parameters of the two diastereoisomers *syn* and *anti*. The predominant metabolic pathway for isopyrazam and its *N*-demethylated metabolite is hydroxylation in

Table 30. Changes in enzyme induction and DNA synthesis in cultured primary female rat hepatocytes

Treatment	% change									
	Control	Phenobarbital sodium salt			CSCD459488					
	Concentration (µmol/l)									
	0	10	100	1000	1	3	10	30	100	500
ATP[a]	—	99.1 ± 5.2	101.8 ± 5.2	132.1 ± 7.2**	113.8 ± 6.1**	115.5 ± 5.2**	111.4 ± 7.1*	121.7 ± 5.3**	129.3 ± 4.1**	2.3 ± 0.2**
DNA synthesis[b]	—	136.8 ± 30.6*	181.8 ± 12.4**	185.3 ± 12.9**	136.9 ± 21.7*	176.5 ± 27.9**	168.0 ± 9.4**	162.3 ± 16.9**	81.3 ± 19.5[c]	[d]
PROD[e]	—	91.4 ± 27.9	497.5 ± 57.5**	567.5 ± 59.7**	257.3 ± 16.8**	424.2 ± 42.6**	527.8 ± 35.3**	561.7 ± 37.4**	69.5 ± 30.8[c]	[d]
BROD[e]	—	138.6 ± 30.7	347.7 ± 71.6**	456.7 ± 48.7**	297.4 ± 20.7**	392.3 ± 77.4**	400.8 ± 67.5**	342.9 ± 4.2**	108.5 ± 7.3[c]	[d]

From Elcombe (2011b)

ATP, adenosine-5′-triphosphate; BROD, benzyloxyresorufin *O*-debenzylase; DNA, deoxyribonucleic acid; PROD, 7-pentoxyresorufin *O*-dealkylase; * $P < 0.05$; ** $P < 0.01$

[a] $n = 6$ per group.

[b] $n = 5$ per group.

[c] No statistical analyses performed (visible cytotoxicity).

[d] No analyses performed (excessive cytotoxicity).

[e] $n = 3$ per group.

Table 31. Changes in enzyme induction and DNA synthesis in cultured primary human female hepatocytes

Treatment	% change relative to control									
	Control	Phenobarbital sodium salt			CSCD459488					
	Concentration (µmol/l)									
	0	10	100	1000	1	3	10	30	100	500
ATP[a]	—	78.5 ± 15.3*	87.0 ± 8.6	78.0 ± 8.0*	79.0 ± 5.9	86.3 ± 3.2	90.1 ± 7.4	79.2 ± 1.8*	28.7 ± 1.8**	0.7 ± 0.1**
DNA synthesis[b]	—	84.9 ± 24.3	102.2 ± 40.9	91.3 ± 22.3	92.6 ± 27.5*	130.2 ± 35.2	120.2 ± 52.6	65.7 ± 12.5	[c]	[c]
PROD[d]	—	241.2 ± 28.0**	235.4 ± 57.3*	623.8 ± 218.2*	218.6 ± 54.5*	201.4 ± 30.4**	187.0 ± 107.0	222.0 ± 48.7*	[c]	[c]
BROD[d]	—	133.7 ± 19.8	221.5 ± 34.4*	555.6 ± 65.5**	120.4 ± 19.5	200.4 ± 34.5*	232.4 ± 113.6	496.7 ± 91.0**	39.1 ± 9.1[e]	[c]

From Elcombe (2011a)

ATP, adenosine-5′-triphosphate; BROD, benzyloxyresorufin *O*-debenzylase; DNA, deoxyribonucleic acid; PROD, 7-pentoxyresorufin *O*-dealkylase; * $P < 0.05$; ** $P < 0.01$

[a] $n = 6$ per group.

[b] $n = 5$ per group.

[c] No analyses performed (excessive cytotoxicity).

[d] $n = 3$ per group.

[e] No statistical analyses performed (excessive cytotoxicity).

the bicyclic isopropyl moiety, followed by further oxidation to form the carboxylic acid or to give rise to multiple hydroxyl moieties, with subsequent formation of glucuronic acid or sulfate conjugates. The structure of isopyrazam provides the potential for stereoisomerization of most metabolites.

Toxicological data

Isopyrazam technical with a *syn*:*anti* ratio of up to 69.7:30.3 is of low acute oral toxicity. The oral LD_{50} was greater than 2000 mg/kg bw in female rats. However, as the oral LD_{50} of pure *anti* isomer or of a 1:1 *syn*:*anti* batch was 310.2 mg/kg bw in female rats, there seems to be an isomeric difference in toxicity at very high dose levels. By dermal application, the LD_{50} of a 92.8:7.2 *syn*:*anti* batch was greater than 5000 mg/kg bw, and the LC_{50} in an inhalation study was greater than 5.28 mg/l. Isopyrazam was not irritating to the skin and only initially slightly irritating to the eye. Isopyrazam showed skin sensitizing potential in a mouse local lymph node assay.

In repeated-dose studies in mice, rats and dogs, the main effects were changes in clinical chemistry (plasma protein, cholesterol, triglycerides, liver enzymes) and haematological parameters (red blood cell counts, haemoglobin, haematocrit), effects on the liver (hepatocellular hypertrophy) and body weight changes.

In a 13-week mouse feeding study with dietary concentrations up to 7000 ppm, reduced body weight in spite of higher feed consumption was observed. Red blood cell parameters were reduced and platelet counts were increased, particularly at the high dose. In females, a few clinical chemistry parameters were elevated. Relative liver weights were increased, and hepatocellular hypertrophy was observed. For one male and one female high-dose animal, necrotic liver nodules were noted. The NOAEL was 2500 ppm (equal to 390.8 mg/kg bw per day), based on haematological changes at 7000 ppm (equal to 1328.8 mg/kg bw per day).

In a study to compare the toxicity of the *syn* and *anti* conformation isomers of isopyrazam, pure *syn* epimer, pure *anti* epimer and 1:1 *syn*:*anti* epimer were administered to rats at up to 5000 ppm for 4 weeks in the diet. With pure *anti* and 1:1 *syn*:*anti*, feed intake and body weight were reduced. Haematological and clinical chemistry parameters were affected with all three compounds, but more severely with pure *anti* and 1:1 *syn*:*anti*. Liver weights were increased in females in all dosed groups and in males at 2000 ppm and above. The Meeting considered the liver findings as adaptive effects and not toxicologically relevant. All compounds in both sexes mildly elevated the total hepatic CYP content and mildly increased EROD (CYP1A) activity, but markedly increased PROD (CYP2B) activity. The NOAEL for pure *syn* was 2000 ppm (equal to 176.7 mg/kg bw per day), based on reduced body weight and increased cholesterol levels at 5000 ppm (equal to 437.3 mg/kg bw per day). The NOAEL for pure *anti* and 1:1 *syn*:*anti* was 500 ppm (equal to 45.2 mg/kg bw per day), based on reduced body weight gain and increased cholesterol levels at 2000 ppm (equal to 176.7 mg/kg bw per day). In two further 4-week rat feeding studies with slightly different isopyrazam batches (*syn*:*anti* ratios of 89:11 and 92.8:7.2), the NOAELs were 500 ppm (equal to 46.1 mg/kg bw per day) and 300 ppm (equal to 28.1 mg/kg bw per day), based on body weight changes, clinical chemistry and haematological changes at 2000 ppm (equal to 174.9 mg/kg bw per day) and above.

In a 13-week rat feeding study with dietary concentrations of isopyrazam (92.8:7.2 *syn*:*anti*) of up to 6000 ppm, reduced feed consumption and body weight gain were noted. Relative liver weight increases were accompanied by hepatocellular hypertrophy. Relative brain weights were decreased in both sexes at the highest dose level. Triglyceride and bilirubin levels were decreased at 1500 ppm and above. The NOAEL was 300 ppm (equal to 21.3 mg/kg bw per day), based on clinical chemistry changes at 1500 ppm (equal to 106.3 mg/kg bw per day). In a 13-week rat feeding study with dietary concentrations of isopyrazam (97.7:2.3 *syn*:*anti*) of up to 4000 ppm, the NOAEL was 1000 ppm (equal to 74.1 mg/kg bw per day), based on significantly increased relative liver weights and hepatocellular hypertrophy in males and females at 4000 ppm (equal to 314.8 mg/kg bw per day).

In a comparative 13-week rat feeding study with dietary concentrations of two batches of isopyrazam (*syn*:*anti* ratios 92.8:7.2 and 69.7:30.3) up to 2000 ppm, the NOAEL for both compounds was 250 ppm (equal to 20.5 mg/kg bw per day), based on body weight effects and hepatocellular hypertrophy and vacuolation at 2000 ppm (equal to 161.0 mg/kg bw per day).

In dogs, two 13-week gelatine capsule gavage studies were performed with two batches of isopyrazam (*syn*:*anti* ratios 92.8:7.2 and 69.7:30.3). In the first study with isopyrazam *syn*:*anti* 92.8:7.2, several behavioural changes, reduced feed consumption and body weight gain, changes in clinical chemistry parameters and increases in liver weights were noted. The NOAEL in this study was 30 mg/kg bw per day, based on behavioural changes and liver weight increases at 100 mg/kg bw per day. In the second study with isopyrazam *syn*:*anti* 69.7:30.3, the NOAEL was 30 mg/kg bw per day, based on clinical observations and initial body weight loss at 250 mg/kg bw per day. In a 52-week gelatine capsule gavage study with isopyrazam (*syn*:*anti* 92.8:7.2), no clinical signs were observed. Initially reduced feed consumption and lower body weights were noted throughout the study. Some clinical chemistry parameters showed occasional modest changes but were without any histopathological correlates or other signs of toxicity. The NOAEL in this study was 25 mg/kg bw per day, based on changes in clinical chemistry parameters and in liver weight at higher dose levels. The overall NOAEL for the effects of isopyrazam with a *syn*:*anti* ratio down to 69.7:30.3 in the 3-month and 1-year studies in dogs was 30 mg/kg bw per day.

In an 18-month feeding study in mice with dietary concentrations of isopyrazam (*syn*:*anti* 92.8:7.2) up to 3500 ppm, the incidence of males with eye discharge was elevated at 3500 ppm. Also at 3500 ppm, body weight gain was reduced in both sexes, body weight–adjusted spleen weights were decreased and liver weights were increased. At 500 ppm, the incidence of periportal hepatocellular hypertrophy in females was increased. At 3500 ppm, the incidences of epithelial eosinophilic droplets in the nasal cavity of males and in the gall bladder of females were elevated. The incidences of benign or malignant tumours were not increased at any dose. The NOAEL was 70 ppm (equal to 9.9 mg/kg bw per day), based on periportal hepatocellular hypertrophy in females at 500 ppm (equal to 74.9 mg/kg bw per day).

Isopyrazam was not carcinogenic in mice.

In a 104-week feeding study in rats with dietary concentrations up to 3000 ppm isopyrazam (*syn*:*anti* 92.8:7.2), body weight gain was decreased in all dosed females and in high-dose males. In both sexes, haematological parameters were changed and the prothrombin time was reduced, and in females, the activated partial thromboplastin time was prolonged at 500 ppm and above. There were changes in some clinical chemistry parameters (e.g. urea, triglyceride and bilirubin levels in females) in all dose groups. At terminal kill, relative brain weights in females at 3000 ppm were increased, and liver weights in both sexes were increased at 500 and 3000 ppm. Adrenal weights in females were decreased at 3000 ppm. The incidences of foci of eosinophilic hepatocytes were increased statistically significantly at 500 ppm and above in both sexes. The hepatocellular pigmentation in all dosed females and in high-dose males was not considered to be of toxicological relevance at the lowest dose because it was of minimal severity. The increased centrilobular hepatocellular hypertrophy observed at all dose levels in both sexes was considered to represent adaptive changes and not to be of toxicological significance. In females at 3000 ppm, there was an increase in hepatocellular adenoma (17%), and at 3000 ppm, one hepatocellular carcinoma was found in each sex. In the high-dose females, the incidence of uterine endometrial adenocarcinoma was increased (23%). The NOAEL was 100 ppm (equal to 5.5 mg/kg bw per day), based on reduced body weight gain in females and increased incidences of foci of eosinophilic hepatocytes and clinical chemistry changes of equivocal toxicological significance in both sexes at 500 ppm (equal to 27.6 mg/kg bw per day).

Isopyrazam was carcinogenic in female rats at the highest dose tested.

The potential genotoxicity of isopyrazam was tested in an adequate range of in vitro and in vivo studies, providing no evidence of genotoxic potential.

The Meeting concluded that isopyrazam is unlikely to be genotoxic.

In mechanistic studies to evaluate possible modes of action for liver tumours in female rats, isopyrazam was shown to induce CYP2B and CYP3A activities and replicative deoxyribonucleic acid (DNA) synthesis in female rat hepatocytes in vitro with a significantly higher potency than phenobarbital. CYP3A was also induced with a high potency in female human hepatocytes in vitro, whereas phenobarbital showed a weak induction potential. In a 14-day feeding study in rats, CYP1A- and CYP2B-dependent activities were induced significantly at all three time points (3, 8 and 15 days after treatment with 500 or 3000 ppm), whereas CYP3A- and CYP4A-dependent activities were not induced. The significant in vivo CYP1A induction and the very high potency CYP2B (in vitro and in vivo) and CYP3A induction (in vitro) for isopyrazam suggest more than phenobarbital-like enzyme induction. Although microsomal enzyme induction was observed, no clear mode of action was identified that could be causally linked to the liver adenoma in female rats.

In an in vitro test with hERα, isopyrazam did not show significant binding capacity. Isopyrazam was negative in a rat uterotrophic assay. Therefore, an estrogen-like mode of action as a possible explanation for the uterine endometrial adenocarcinoma is not supported.

On the basis of the absence of genotoxicity and the absence of carcinogenicity in mice and the fact that an increase in the incidences of hepatocellular adenoma and uterine endometrial adenocarcinoma in female rats occurred only at the highest dose tested, the Meeting concluded that isopyrazam is unlikely to pose a carcinogenic risk to humans at dietary exposure levels.

In a two-generation study of reproductive toxicity in rats at dietary concentrations up to 3000 ppm isopyrazam (*syn*:*anti* 92.8:7.2), F_0 and F_1 rats had decreased body weight at 500 and 3000 ppm. Hepatocellular hypertrophy and increases in liver weights were noted in F_0 and F_1 animals at 500 ppm and above. In F_0 males at 500 ppm and above, thyroid weights were increased statistically significantly. In F_0 and F_1 females at 3000 ppm, weights of the uterus with cervix were decreased statistically significantly. Kidney weights in F_1 animals were dose-relatedly increased at all dose levels in both sexes, statistically significantly in females at all dose levels and in males at 3000 ppm. Ovary weights in high-dose F_0 and F_1 females were statistically significantly decreased. F_{1A} and F_{2A} pup body weights were reduced at 3000 ppm. At 500 ppm and above, mean total litter weights were reduced. F_1 males at 3000 ppm showed statistically significantly delayed preputial (2.3 days) separation, and F_1 females at 3000 ppm showed statistically significantly delayed vaginal opening (2 days). Whereas the males showed statistically significantly reduced body weights (−7%), the body weights of females were unchanged. The NOAEL for parental toxicity was 100 ppm (equal to 8.1 mg/kg bw per day), based on decreased body weight gain and organ weight changes at 500 ppm (equal to 40.6 mg/kg bw per day) in parental F_0 and F_1 animals. The NOAEL for postnatal developmental toxicity was 100 ppm (equal to 8.1 mg/kg bw per day), based on decreased mean total litter weights at 500 ppm (equal to 40.6 mg/kg bw per day). The NOAEL for reproductive performance was 3000 ppm (equal to 239.1 mg/kg bw per day), the highest dose tested.

In a study on the developmental toxicity of isopyrazam (*syn*:*anti* 92.8:7.2) in rats at dose levels up to 250 mg/kg bw per day administered by gavage, two high-dose dams were killed in extremis on GDs 20 and 21 because they were showing severe signs of toxicity. The high dose group animals showed reduced feed consumption and reduced body weight, and fetal body weights were decreased in this group. In the 250 mg/kg bw per day group, one fetus with hydroencephalus and microphthalmia was observed, and in another litter, a fetus with hydrocephalus only was noted. Non-ossified cervical centra and incomplete xiphoid cartilage were noted at 75 mg/kg bw per day and above. The NOAEL for maternal toxicity was 75 mg/kg bw per day, based on reduced body weight and clinical signs of toxicity at 250 mg/kg bw per day. The NOAEL for developmental toxicity was 20 mg/kg bw per day, based on non-ossified cervical centra at 75 mg/kg bw per day.

In a study on the developmental toxicity of isopyrazam (*syn*:*anti* 69.7:30.3) in rats at dose levels up to 200 mg/kg bw per day administered by gavage, ventral recumbency and sedation were noted

in all dams at 200 mg/kg bw per day, from the 1st day of treatment (GD 4) throughout the 1st week. Feed consumption and body weight gain were reduced from GD 4 in animals at 75 mg/kg bw per day and above. At 75 mg/kg bw per day and above, fetal body weights were lower than those of controls. One fetus at 200 mg/kg bw per day was found with diaphragmatic hernia. Increased incidences of delayed or absent ossification of cervical vertebral bodies were observed at 200 mg/kg bw per day and of incompletely ossified sternebrae at 75 mg/kg bw per day and above. Additionally, non-ossified structures in forelimbs and hindlimbs were identified. The NOAEL for maternal and developmental toxicity was 20 mg/kg bw per day, based on clinical signs and reduced body weight gain in dams and lower fetal body weights at 75 mg/kg bw per day.

In two range-finding studies on the developmental toxicity of isopyrazam (*syn*:*anti* 92.8:7.2) in Himalayan rabbits at dose levels up to 1000 mg/kg bw per day, no maternal toxicity was observed. In fetuses, the incidences of small eyes, malrotated and flexed limbs and changes in the skull were increased at 400 mg/kg bw per day and above.

In a third range-finding study on the developmental toxicity of isopyrazam (*syn*:*anti* 92.8:7.2) in New Zealand White rabbits at dose levels up to 1000 mg/kg bw per day, maternal toxicity was noted at all dose levels from 400 to 1000 mg/kg bw per day, as dams had decreased body weight, increased relative liver weights, hepatocellular hypertrophy and changes in clinical chemistry parameters. At the high dose, fetal body weight was reduced and early resorptions were increased. Small eyes were noted in fetuses at 1000 mg/kg bw per day. Furthermore, absent gall bladders, extra papillary muscle in the heart and variations of major blood vessels were observed at all dose levels.

In a definitive study on the developmental toxicity of isopyrazam (*syn*:*anti* 92.8:7.2) in New Zealand White rabbits at dose levels up to 500 mg/kg bw per day, hepatocellular vacuolation was observed at 500 mg/kg bw per day. One fetus in the 500 mg/kg bw per day group had bilateral microphthalmia. The NOAEL for maternal toxicity was 150 mg/kg bw per day, based on a statistically significantly increased incidence of centrilobular vacuolation at 500 mg/kg bw per day. The developmental NOAEL was 150 mg/kg bw per day, based on a single observation of bilateral microphthalmia at 500 mg/kg bw per day.

A low incidence of microphthalmia was consistently observed in dose range–finding and main studies in two different rabbit strains. Microphthalmia is a very rare finding in the rabbit strains used. Thus, the Meeting concluded that the low incidences of microphthalmia in treated rabbits could not be discounted.

The Meeting concluded that isopyrazam was teratogenic in rabbits.

In an acute neurotoxicity study in rats administered isopyrazam (*syn*:*anti* 92.8:7.2) at doses ranging from 30 to 2000 mg/kg bw, nonspecific and transient effects were apparent within 3 hours after dosing in all dose groups, with a dose-dependent increase in the incidence and severity of rigidity. The NOAEL for systemic toxicity was 30 mg/kg bw, based on clinical signs of toxicity at 250 mg/kg bw. The NOAEL for acute neurotoxicity was 2000 mg/kg bw, the highest dose tested.

In a 13-week rat feeding study of the neurotoxicity of isopyrazam (*syn*:*anti* 92.8:7.2) with dietary concentrations up to 6000 ppm, no behavioural or histological evidence for neurotoxicity was observed. The NOAEL for systemic toxicity was 1500 ppm (equal to 98.01 mg/kg bw per day), based on decreased body weight gain in females at 6000 ppm (equal to 382.26 mg/kg bw per day). The NOAEL for subchronic neurotoxicity was 6000 ppm (equal to 382.26 mg/kg bw per day), the highest dose tested.

Toxicological data on metabolites

CSCD465008, a soil and plant metabolite, and CSCD459488, a rat, soil, plant and aquatic metabolite, were investigated in acute and short-term toxicity studies and an adequate range of in

vitro genotoxicity studies. CSCD459488 was also investigated in a developmental toxicity study in rabbits.

CSCD465008 and CSCD459488 were both of low acute oral toxicity, with LD_{50} values greater than 2000 mg/kg bw, and did not give any evidence of genotoxic potential.

In a 4-week rat feeding study with dietary concentrations of CSCD465008 up to 12 000 ppm, no evidence for toxicity or for induction of EROD or PROD activity was observed. The NOAEL was 12 000 ppm (equal to 1018 mg/kg bw per day), the highest dose tested.

In a 4-week rat feeding study with dietary concentrations of CSCD459488 up to 10 000 ppm, the relative liver weights were increased at 300 ppm and above, and increased incidences of centrilobular hepatocyte hypertrophy and follicular cell hypertrophy of the thyroid were noted at 4000 ppm and above. Total hepatic microsomal CYP content was approximately doubled in males in the 4000 and 10 000 ppm groups and only slightly elevated in females in the same dose groups. EROD and PROD activities were statistically significantly increased at all dose levels. The NOAEL was 300 ppm (equal to 27 mg/kg bw per day), based on liver weight changes greater than 10% at higher doses.

In a study on the developmental toxicity of CSCD459488 in New Zealand White rabbits at dose levels up to 1000 mg/kg bw per day, relative maternal liver weights were increased at all dose levels. In the high-dose group, late resorptions per litter were increased primarily due to three resorptions in one female. The maternal NOAEL was 150 mg/kg bw per day, based on significant liver weight increases (> 20%) at higher dose levels. The NOAEL for developmental toxicity was 1000 mg/kg bw per day, the highest dose tested.

No reports on exposure of personnel working with isopyrazam were submitted.

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

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) of 0–0.06 mg/kg bw, derived from the NOAEL of 5.5 mg/kg bw per day in the 104-week rat feeding study on the basis of decreased body weight gain in females and increased incidences of foci of eosinophilic hepatocytes and clinical chemistry changes (triglycerides, bilirubin) of equivocal toxicological significance in both sexes at 27.6 mg/kg bw per day. A safety factor of 100 was applied. The ADI is supported by the NOAEL of 9.9 mg/kg bw per day in the mouse 80-week feeding study, based on periportal hepatocellular hypertrophy in females at 56.2 mg/kg bw per day. The margin between the maximum ADI and the lowest-observed-adverse-effect level (LOAEL) at 232.8 mg/kg bw per day for uterine and liver tumours in female rats is approximately 3900.

The Meeting established an acute reference dose (ARfD) of 0.3 mg/kg bw, derived from the NOAEL of 30 mg/kg bw in the rat acute neurotoxicity study, on the basis of nonspecific clinical signs of toxicity (weak appearance and decreased activity) at 250 mg/kg bw. A safety factor of 100 was applied. In a rat developmental toxicity study, the NOAEL of 20 mg/kg bw per day for maternal and developmental toxicity was based on reduced body weight gain in dams only on day 4 of treatment. The margin between the ARfD and the LOAEL at 500 mg/kg bw per day for teratogenic effects (microphthalmia) in rabbits is approximately 1700.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen-month study of toxicity and carcinogenicity[a]	Toxicity	70 ppm, equal to 9.9 mg/kg bw per day	500 ppm, equal to 56.2 mg/kg bw per day
		Carcinogenicity	3500 ppm, equal to 432.6 mg/kg bw per day[b]	—
Rat	Acute neurotoxicity[c]	Toxicity	30 mg/kg bw	250 mg/kg bw
	Two-year study of toxicity and carcinogenicity[a]	Toxicity	100 ppm, equal to 5.5 mg/kg bw per day	500 ppm, equal to 27.6 mg/kg bw per day
		Carcinogenicity	500 ppm, equal to 34.9 mg/kg bw per day	3000 ppm, equal to 232.8 mg/kg bw per day
	Two-generation study of reproductive toxicity[a]	Reproductive toxicity	3000 ppm, equal to 239.1 mg/kg bw per day[b]	—
		Parental toxicity	100 ppm, equal to 8.1 mg/kg bw per day	500 ppm, equal to 40.6 mg/kg bw per day
		Offspring toxicity	100 ppm, equal to 8.1 mg/kg bw per day	500 ppm, equal to 40.6 mg/kg bw per day
	Developmental toxicity study[c]	Maternal toxicity	20 mg/kg bw per day	75 mg/kg bw per day
		Embryo and fetal toxicity	20 mg/kg bw per day	75 mg/kg bw per day
Rabbit	Developmental toxicity study[c]	Maternal toxicity	150 mg/kg bw per day	500 mg/kg bw per day
		Embryo and fetal toxicity	150 mg/kg bw per day	500 mg/kg bw per day
Dog	Thirteen-week and 1-year studies of toxicity[c,d]	Toxicity	30 mg/kg bw per day	100 mg/kg bw per day

[a] Dietary administration.
[b] Highest dose tested.
[c] Gavage administration.
[d] Three studies combined.

Estimate of acceptable daily intake for humans

0–0.06 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 exposures

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

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid, 70%
Dermal absorption	No data
Distribution	Extensive, highest levels in liver

Potential for accumulation	Low, no evidence of accumulation
Rate and extent of excretion	Rapid, close to 100% within 48 h, mainly via bile
Metabolism in animals	Extensive, primarily via hydroxylation at bicyclic-isopropyl moiety
Toxicologically significant compounds (animals, plants and the environment)	Isopyrazam, CSCD459488

Acute toxicity

Rat, LD_{50}, oral	> 2000 mg/kg bw (69.7:30.3 *syn*:*anti*)
	310.2 mg/kg bw (1:1 *syn*:*anti* and pure *anti*)
Rat, LD_{50}, dermal	> 5000 mg/kg bw (92.8:7.2 *syn*:*anti*)
Rat, LC_{50}, inhalation	> 5.28 mg/l (69.7:30.3 *syn*:*anti*)
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Slightly irritating
Mouse, skin sensitization (local lymph node assay)	Sensitizing potential

Short-term studies of toxicity

Target/critical effect	Body weight changes and liver toxicity (rat)
Lowest relevant oral NOAEL	20.3 mg/kg bw per day (rat)
Lowest relevant dermal NOAEL	No data
Lowest relevant inhalation NOAEC	No data

Genotoxicity

Not genotoxic

Long-term studies of toxicity and carcinogenicity

Target/critical effect	Clinical chemistry, body weight (rat)
Lowest relevant NOAEL	5.5 mg/kg bw per day (rat)
Carcinogenicity	Unlikely to pose a carcinogenic risk at dietary exposure levels

Reproductive toxicity

Reproduction target/critical effect	No reproductive effects
Lowest relevant reproductive NOAEL	239.1 mg/kg bw per day (rat), highest dose tested
Developmental target/critical effect	Decreased fetal body weights (rat), microphthalmia (rabbit)
Lowest relevant developmental NOAEL	20 mg/kg bw per day (rat), 150 mg/kg bw per day (rabbit)

Neurotoxicity/delayed neurotoxicity

No evidence in acute or subchronic neurotoxicity studies

Other toxicological studies

Studies on metabolites	In rat 4-week feeding studies, CSCD465008 was less toxic than the parent and CSCD459488 was of similar toxicity to the parent

Medical data

No reports submitted

Summary

	Value	Study	Safety factor
ADI	0–0.06 mg/kg bw	Two-year study in rats	100
ARfD	0.3 mg/kg bw	Acute neurotoxicity study in rats	100

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PENTHIOPYRAD

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Explanation

Penthiopyrad is the International Organization for Standardization (ISO)–approved name for *N*-[2-(1,3-dimethylbutyl)-3-thienyl]-1-methyl-3-(trifluoromethyl)-1H-pyrazole-4-carboxamide (9CI) (Chemical Abstracts Service No. 183675-82-3). It is a new 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. Penthiopyrad 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 pivotal 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 penthiopyrad have been investigated in Wistar Hanover rats. Summaries of the relevant data are presented below.

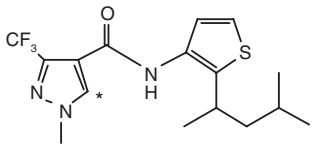
1.1 Absorption, distribution and excretion

In an oral metabolism study, Wistar Hanover rats were administered a single oral (gavage) dose of penthiopyrad at either 10 or 100 mg/kg body weight (bw) in a vehicle of aqueous 10% Tween 80 and evaluated according to the following protocols. All experiments in this study were replicated with each of the two radiolabelled forms: ^{14}C-pyrazole-ring-labelled penthiopyrad, or P-label (97% radiochemical purity); or ^{14}C-thienyl-ring-labelled penthiopyrad, or T-label (98.7% radiochemical purity) (Figure 1). For the main excretion study, urine, faeces and cage washes were collected from four rats of each sex per treatment group for each radiolabel at 6, 12, 24, 48, 72 and 96 hours, and the rats were terminated at 96 hours. For the study of blood kinetics, blood was taken via a jugular cannula from four rats of each sex per treatment group for each radiolabelled form at several time points up to 72 hours after dosing, when the animals were terminated. For the study of biliary excretion, four rats of each sex per treatment group and radiolabelled form were dosed. Bile was sampled up to 72 hours after dosing. For the study of tissue distribution, groups of three rats of each sex per treatment group and radiolabel per time point (total of 24 rats for each radiolabelled form) were terminated at 1, 24, 48 and 72 hours after treatment. A wide range of major organs and tissues were excised and assayed for radiolabel content (Gupta et al., 2009). In a repeated-dose metabolism study, four groups of three Wistar Hanover rats of each sex per treatment group were treated once daily, by gavage, with [^{14}C-pyrazole]penthiopyrad, or P-label (radiochemical purity > 97% active ingredient [ai]), in 10% aqueous Tween 80 vehicle at a dose level of 10 mg/kg bw per day (approximately 2.8 MBq/kg bw per day in 4 ml vehicle per kilogram body weight). The purpose of the study was to evaluate the influence of multiple dosing on the oral absorption, distribution, excretion and metabolism of [^{14}C-pyrazole]penthiopyrad in rats and to compare these data with results from a single-dose gavage metabolism study. One group, treated for 7 days and sacrificed 96 hours after the final dose, was used to investigate excretion, mass balance and metabolite profile in urine and faeces. One group treated for 4 days and sacrificed 24 hours after the final dose was used to investigate the metabolic profile in plasma. Two further groups, treated for 7 days and sacrificed either 24 or 72 hours after the final dose, were used to investigate the metabolic profile in plasma and the distribution in selected tissues (target organs and tissues showing a tissue:plasma concentration ratio > 1 in the single-dose study) (Shah & McClanahan, 2009).

Recovery of radioactivity in the excretion studies exceeded 91% of the administered dose for all groups evaluated (Table 1). The major portion of the administered dose was excreted in the faeces (69.6–84.3% of the administered dose), with a smaller but significant amount (7.3–21.8% of the administered dose) excreted in the urine (Tables 1 and 2). Excretion was rapid in both sexes for

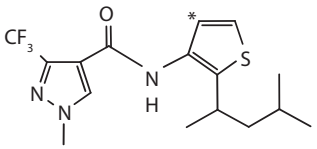
Figure 1. Radiolabelled forms of penthiopyrad used in absorption, distribution, metabolism and excretion studies: structure and position of labels

Structural formula of [^{14}C-pyrazole]penthiopyrad (P-label)



* position of label

Structural formula of [^{14}C-thienyl]penthiopyrad (T-label)



* position of label

Table 1. Comparison of recovery of radiolabel after single[a] and multiple doses[b] of [^{14}C]penthiopyrad at 10 mg/kg bw per day in rats

Matrix	% of administered dose			
	Males		Females	
	Single dose	Multiple doses	Single dose	Multiple doses
Urine	13.24	17.15	21.79	22.77
Cage wash	1.24	2.28	1.81	3.11
Faeces	77.06	71.84	69.61	64.98
Gastrointestinal tract + contents	0.06	0.03	0.05	0.03
Whole blood	NA	0.04	NA	0.03
Carcass	0.26	0.23	0.24	0.19
Total	91.86	91.58	93.50	91.11

NA, not available

[a] Data for P-label, 10 mg/kg bw, derived from Gupta et al. (2009).

[b] Data for P-label, 10 mg/kg bw per day for 7 days, derived from Shah & McClanahan (2009).

all treatment regimes, with 74.8–85.0% of the administered dose being eliminated within 24 hours. From the experiment with bile duct–cannulated rats, the overall systemic absorption from the gastrointestinal tract was calculated to be in the range 83.9–91.9% of the administered dose based on the 72-hour data (Table 2). There were no significant sex- or dose-related differences in the overall systemic absorption or excretion for either label, although females showed a slightly higher area under the curve (AUC) (see Table 3 below) and urinary excretion compared with males. Multiple dosing did not alter the excretion pattern.

The plasma concentrations of both radiolabels reached their maximal level by 0.5 hour post-dosing (T_{max}) for the 10 mg/kg bw groups and by 1.3 hours post-dosing for the 100 mg/kg bw groups. The maximum plasma concentrations (C_{max}) and the AUC were directly proportional to the dose level, indicating linear oral absorption in the dose range 10–100 mg/kg bw. As noted above, females

Table 2. Average recovery of radioactivity in bile duct–cannulated rats 72 hours after a single oral dose of [^{14}C]penthiopyrad

Matrix	% of administered dose			
	10 mg/kg bw		100 mg/kg bw	
	Males	Females	Males	Females
P-label				
Bile	66.64	65.70	74.62	65.69
Faeces	12.19	13.25	9.69	12.93
Urine	13.84	18.27	14.88	17.02
Cage wash	2.19	1.91	2.06	4.23
Gastrointestinal tract + contents	0.09	0.03	0.04	0.04
Carcass	1.20	0.22	0.35	0.84
Total	96.15	99.38	101.64	100.75
Absorbed dose[a]	83.87	86.10	91.91	87.78
T-label				
Bile	70.94	74.31	81.07	62.80
Faeces	8.34	10.23	7.97	11.23
Urine	12.77	8.39	5.02	16.75
Cage wash	2.06	2.66	2.31	6.00
Gastrointestinal tract + contents	0.04	0.17	0.07	0.06
Carcass	0.82	0.61	0.52	0.73
Total	94.97	96.37	96.96	97.57
Absorbed dose[a]	86.59	85.97	88.92	86.28

From Gupta et al. (2009)
[a] Absorbed dose = bile + urine + cage wash + carcass.

Table 3. Selected pharmacokinetic parameters in rats following oral administration of [^{14}C]-penthiopyrad (n = 4)

Sex	Dose (mg/kg bw)	Label position	T_{max} (h)	C_{max} (µg eq/g)	$AUC_{0-\infty}$ (µg eq·h/g)	$t_{½}$ (h)
Male	10	Pyrazole	0.4	1.6	21.9	15.0
Female			0.4	3.3	27.8	13.6
Male	100		1.1	15.2	228.6	16.1
Female			1.3	28.4	322.2	16.8
Male	10	Thienyl	0.5	1.5	21.4	20.0
Female			0.4	3.4	27.4	14.1
Male	100		1.0	14.3	224.8	21.4
Female			1.3	31.9	324.4	17.7

From Gupta et al. (2009)
$AUC_{0-\infty}$, area under the concentration–time curve from zero to infinity; C_{max}, maximum concentration in plasma; eq, equivalent; $t_{½}$, half-life; T_{max}, time to reach maximum concentration in plasma

showed a slightly higher AUC compared with males. Elimination from the blood followed first-order kinetics and was independent of dose level, sex or radiolabel, with half-lives in the range 13.6–21.4 hours (Table 3).

Levels of radioactivity in 31 matrices were measured at 1, 24, 48 and 72 hours after dosing for both dose levels, sexes and radiolabels. The example table (Table 4) is given for male rats dosed with P-label penthiopyrad. Maximum tissue concentrations were observed within 1 hour of dosing, at which time the concentrations in liver, fat, lymph nodes, adrenals, ovaries, pancreas, kidneys, urinary bladder and gastrointestinal tract were higher than those in plasma. Twenty-four hours after dosing, only the concentrations in liver and kidney (multiple doses only) were higher than those in plasma (Table 5). A similar distribution pattern was observed for female rats and for males and females dosed with the thienyl label. Concentrations in all tissues decreased from the maximum levels in the 1-hour samples, with calculated half-lives ranging from 3.2 to 24.7 hours for both dose levels, sexes and radiolabels. The half-life of radioactivity in blood and packed red blood cells ranged from 21.8 to 46.4 hours and from 33.5 to 390.1 hours, respectively. In tissues taken 72 hours after dosing, levels of radioactivity were generally very low. The tissue with the highest concentration was liver, with concentrations in the range 0.14–0.323 part per million (ppm) at 10 mg/kg bw and 1.1–3.6 ppm at 100 mg/kg bw. After 72 hours, total residues for each label in the gastrointestinal tract + contents were less than or equal to 0.1% of the administered dose, and residues in the carcass were less than or equal to 0.4% of the administered dose (Gupta et al., 2009). Repeated dosing led to a small increase of radioactive residues in adrenals, blood, fat, kidney, liver, lung, lymph nodes, ovaries, pancreas and thyroid compared with the corresponding single dose. In the plasma, the radioactivity reached a plateau of 2.8–3.3 times the single dose and then cleared to levels below the limit of detection by the 2nd day after the cessation of dosing. The overall tissue distribution in both sexes 24 hours after multiple doses was similar to that following a single dose (Shah & McClanahan, 2009).

Table 4. Distribution of radioactivity in tissues of male rats administered P-label

	Concentration (ppm penthiopyrad equivalents)			
	1 h	24 h	48 h	72 h
10 mg/kg bw P-label				
Adrenals	1.096	0.100	0.036	0.022
Bone	0.384	0.057	0.019	0.010
Bone marrow	0.532	0.096	0.025	0.011
Brain	0.322	0.030	0.009	0.006
Carcass	0.544	0.111	0.024	0.017
Eyes	0.311	0.050	0.011	0.006
Fat	5.543	0.084	0.024	0.014
Heart	0.744	0.119	0.050	0.045
Intestine	54.789	1.122	0.069	0.027
Intestinal contents	27.663	2.854	0.094	0.021
Kidney	1.976	0.119	0.040	0.029
Liver	10.747	0.440	0.176	0.228
Lung	0.501	0.066	0.023	0.021
Lymph nodes (mesenteric)	3.501	0.099	0.024	0.015
Muscle	0.505	0.054	0.015	0.010
Packed red blood cells	0.579	0.312	0.268	0.239
Pancreas	1.029	0.098	0.023	0.020
Pituitary	0.444	0.086	0.035	0.015
Plasma	1.158	0.163	0.061	0.057

Table 4 (continued)

	Concentration (ppm penthiopyrad equivalents)			
	1 h	24 h	48 h	72 h
Prostate	0.824	0.173	0.020	0.010
Skin	0.405	0.068	0.025	0.021
Spleen	0.471	0.103	0.042	0.045
Stomach	22.898	0.052	0.012	0.017
Stomach contents	4.309	0.008	0.001	0.000
Testes	0.390	0.057	0.015	0.010
Thymus	0.396	0.065	0.017	0.010
Thyroid	0.563	0.104	0.041	0.034
Urinary bladder	2.601	1.276	0.040	0.027
Whole blood	0.725	0.256	0.163	0.132
100 mg/kg P-label				
Adrenals	18.339	0.847	0.410	0.246
Bone	7.572	0.472	0.211	0.096
Bone marrow	10.368	0.833	0.333	0.139
Brain	5.462	0.329	0.102	0.066
Carcass	10.399	1.367	0.268	0.160
Eyes	4.063	0.394	0.130	0.064
Fat	126.602	0.761	0.268	0.145
Heart	9.411	1.016	0.560	0.387
Intestine	290.349	6.613	0.436	0.238
Intestinal contents	265.081	27.024	1.174	0.271
Kidney	19.708	0.963	0.392	0.249
Liver	139.296	3.617	1.785	1.431
Lung	6.623	0.566	0.300	0.233
Lymph nodes (mesenteric)	68.846	0.692	0.251	0.142
Muscle	7.877	0.498	0.182	0.113
Packed red blood cells	6.453	3.076	3.086	2.673
Pancreas	32.645	0.710	0.261	0.231
Pituitary	6.743	0.769	0.372	0.122
Plasma	13.643	1.376	0.747	0.695
Prostate	24.817	0.759	0.256	0.106
Skin	6.426	0.650	0.288	0.194
Spleen	6.508	0.937	0.504	0.375
Stomach	543.572	0.691	0.133	0.130
Stomach contents	279.688	0.449	0.009	0.000
Testes	5.506	0.513	0.159	0.095
Thymus	5.641	0.527	0.199	0.099
Thyroid	9.420	0.967	0.445	0.334
Urinary bladder	82.231	1.174	0.254	0.281
Whole blood	8.482	2.353	2.008	1.415

From Gupta et al. (2009)

Table 5. Comparison of tissue:plasma concentration ratios 24 hours after single and multiple doses of [^{14}C]penthiopyrad at 10 mg/kg bw per day in rats

Tissue	Tissue:plasma concentration ratio					
	Males			Females		
	1 dose	4 doses (group 2)	7 doses (group 3)	1 dose	4 doses (group 2)	7 doses (group 3)
Adrenals	0.613	0.638	0.586	0.877	0.662	0.630
Fat	0.515	0.354	0.412	0.687	0.420	0.405
Kidney	0.730	1.676	1.936	0.692	1.563	1.056
Liver	2.699	3.275	5.156	2.297	5.597	4.138
Lung	0.405	0.817	0.914	0.405	0.864	1.005
Lymph nodes	0.607	0.310	0.411	0.641	0.184	0.392
Ovaries	—	—	—	0.872	0.391	0.578
Pancreas	0.601	0.461	0.461	0.554	0.450	0.484
Plasma	1.000	1.000	1.000	1.000	1.000	1.000
Red blood cells	1.914	2.013	2.624	1.733	1.468	2.334
Thyroid	0.638	0.591	0.658	0.595	0.655	0.701
Whole blood	1.571	1.576	2.151	1.374	1.374	1.911

From Gupta et al. (2009); Shah & McClanahan (2009)

1.2 Biotransformation

Unchanged penthiopyrad was identified in the faeces of males and females at both dose levels. It was the major ^{14}C component in the faeces of males and females at the dose of 100 mg/kg bw using either label (12.3–30.4% of the administered dose). At 10 mg/kg bw, it ranged from 3.1% to 8.1% of the administered dose. Levels of metabolites showed some quantitative differences between the sexes and dose levels. There were a large number of metabolites (at least 22) in faecal extracts separated by high-performance liquid chromatography (HPLC). Metabolites present at greater than 5% of the administered dose in faeces in at least one dosing regimen were two isomers of DM-A-COOH, two isomers of 753-A-COOH, 753-A-OH (*N*-[2-(3-hydroxy-1,3-dimethyl-butyl) thiophen-3-yl]-1-methyl-3-trifluoromethyl-1H-pyrazole-4-carboxamide), an isomer of DM-A-OH and 1-methyl-3-trifluoromethyl-1H-pyrazole-4-carboxamide (PAM).

In bile, penthiopyrad was present in trace amounts (Table 6). Two major metabolites in bile were identified by liquid chromatography–mass spectrometry (LC-MS) as glucuronide conjugates of isomers of DM-A-OH. Concentrations of each conjugate ranged from 2.1% to 9.9% of the administered dose. Other major metabolites in bile were 753-A-OH (up to 6.75% of the administered dose), 753-A-COOH isomer b (up to 7.4% of the administered dose), DM-A-COOH isomer b (6.0% of the administered dose) and a dehydro 753-A-OH isomer (up to 5.85% of the administered dose). The four metabolites containing the label from the pyrazole moiety—PAM, 3-trifluoromethyl-1H-pyrazole-4-carboxamide (DM-PAM), 1-methyl-3-trifluoromethyl-1H-pyrazole-4-carboxylic acid (PCA) and 3-trifluoromethyl-1H-pyrazole-4-carboxylic acid (DM-PCA)—were present in bile in low amounts, each less than 0.4% of the administered dose. A large number of other minor metabolites of intermediate polarity were separated and quantified by HPLC.

In urine, overall levels of radioactivity were lower than in faeces or bile, so none of the metabolites exceeded 5% of the administered dose. Most abundant were 753-A-COOHb (1.1–4.0% of the administered dose), DM-COOHb (0.4–3.5% of the administered dose), PCA (0.8–2.2% of the

Table 6. Metabolite identity in bile of rats after a single oral dose of [^{14}C]penthiopyrad

Metabolite	Cumulative total (% of the administered dose) at 24 h			
	10 mg/kg bw		100 mg/kg bw	
	Males	Females	Males	Females
Bile (total) / ^{14}C-pyrazole	66.22	65.35	72.97	63.82
Main (total)	27.90	16.64	24.87	18.26
DM-PAM	0.31	0.34	0.07	0.09
PAM	0.39	0.24	0.24	0.19
DM-PCA	0.02	0.02	0.04	0.03
PCA	0.13	0.15	0.13	0.11
Metabolite 42.45min	5.31	2.92	3.50	2.55
DM-A-COOHa	2.13	2.12	0.98	4.80
753-A-COOHa	4.21	2.25	3.09	1.04
Metabolite 44.63min	5.62	1.38	2.07	1.54
DM-A-COOHb	1.46	2.37	5.15	3.70
753-A-COOHb	3.77	1.88	7.37	1.98
753-A-OH	4.07	2.58	1.92	1.84
753-T-DO	0.11	0.08	0.10	0.14
DM-753	0.21	0.22	0.06	0.06
Penthiopyrad (MTF-753)	0.17	0.10	0.16	0.19
Polars (total)	4.93	3.79	2.89	2.90
Three polars	≤ 4.23 each	≤ 2.69 each	≤ 2.63 each	≤ 2.47 each
Conjugates (total)	11.45	16.68	5.99	10.40
Metabolite 38.48min	6.22	8.87	3.26	5.01
Metabolite 39.55min	5.23	7.81	2.73	5.39
Others (total)	21.93	28.24	39.22	32.26
Up to 24 others	≤ 2.82 each	6.47 5.47 ≤ 2.39 each	12.36 ≤ 3.27 each	≤ 3.99 each
Bile (total) / ^{14}C-thienyl	70.70	73.88	79.53	58.62
Main (total)	27.27	16.04	31.72	18.41
Metabolite 42.45min	4.70	2.15	5.14	2.91
DM-A-COOHa	1.95	0.08	4.30	2.62
753-A-COOHa	1.11	0.98	3.72	1.35
Metabolite 44.63min	2.51	3.23	5.85	1.43
DM-A-COOHb	6.01	2.18	2.51	2.79
753-A-COOHb	3.85	2.70	7.06	4.29
753-A-OH	6.75	4.03	2.83	2.82
753-T-DO	0.21	0.27	0.17	0.04
DM-753	0.17	0.25	0.08	0.04
Penthiopyrad (MTF-753)	0.02	0.16	0.05	0.13

Table 6 (continued)

Metabolite	Cumulative total (% of the administered dose) at 24 h			
	10 mg/kg bw		100 mg/kg bw	
	Males	Females	Males	Females
Polars (total)	5.46	3.33	6.55	5.51
Three polars	≤ 4.53 each	≤ 2.04 each	6.11 ≤ 0.22 each	≤ 4.92 each
Conjugates (total)	10.44	18.37	5.54	8.72
Metabolite 38.48min	4.24	9.89	2.14	4.40
Metabolite 39.55min	6.20	8.48	3.40	4.32
Others (total)	27.53	36.14	35.73	25.98
Up to 26 others	≤ 2.78 each	5.96 5.25 ≤ 3.25 each	≤ 3.95 each	≤ 2.08 each

From Sugiyama & Dohn (2009)

administered dose), PAM (0.9–1.7% of the administered dose) and DM-PAM (0.7–1.5% of the administered dose). DM-PCA was observed at a low level (0.5% of the administered dose) in one sample. Liver and red blood cells had a high and rapidly increasing level of bound residues, indicative of extensive metabolism and incorporation of radiolabel (Sugiyama & Dohn, 2009).

The proposed metabolic pathway based on all the rat metabolism studies is shown in Figure 2.

There were no marked differences in the metabolic pathway due to sex or dose level. Two labelling positions were used, which demonstrated that following cleavage, the label from the pyrazole moiety was found in four metabolites: PAM, DM-PAM, PCA and DM-PCA. These metabolites were shown to be released from the bound residue in liver. The fate of the thienyl ring following cleavage was more difficult to elucidate, as no major extractable metabolites were formed. It is assumed from the nature of the metabolites characterized that the ring breaks down completely, with small fragments being distributed widely through intermediary metabolism (Gupta et al., 2009). The same metabolites occurred in plasma from both the single-dose and multiple-dose studies.

2. Toxicological studies

2.1 Acute toxicity

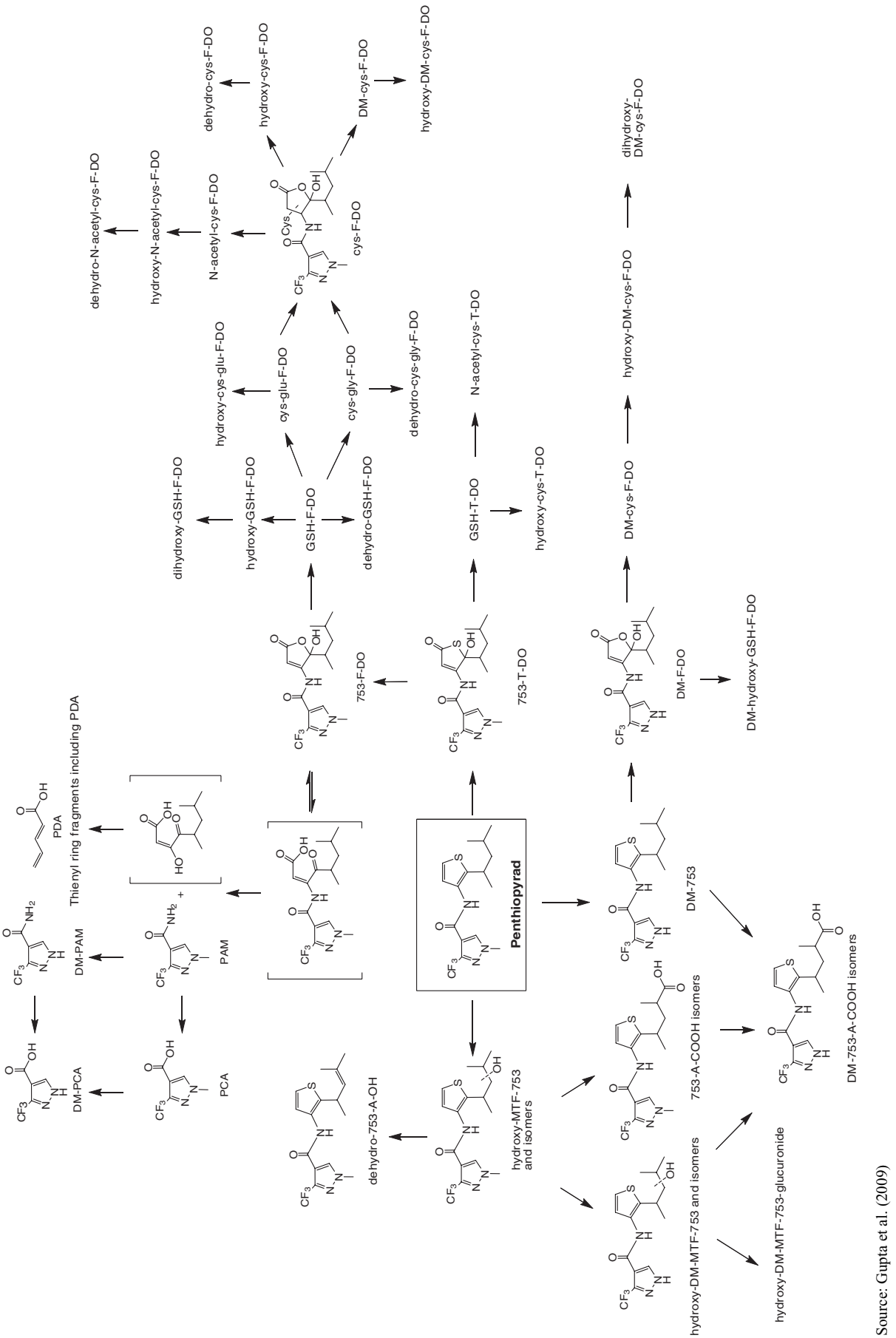
(a) Lethal doses

The results of acute toxicity studies with penthiopyrad administered orally, dermally or by inhalation are summarized in Table 7. All the studies were certified as complying with GLP.

A group of three male and three female young adult Wistar rats was treated once orally, by gavage, with an aqueous suspension of penthiopyrad 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 or clinical signs of an adverse reaction to treatment occurred, and there were no macroscopic findings at necropsy in any animal. One male showed low overall weight gain during the observation period, but the weight gain of other animals was within the expected range. Based on

Figure 2. Proposed metabolic pathway of penthiopyrad in rat



Source: Gupta et al. (2009)

Table 7. Acute toxicity of penthiopyrad

Species	Strain	Sex	Route	Purity (%)	Result	Reference
Rat	Wistar	Male and female	Oral	99.8	LD_{50} > 2000 mg/kg bw	Arcelin (2000)
Rat	Wistar	Male and female	Dermal	99.8	LD_{50} > 2000 mg/kg bw	Arcelin (2001)
Rat	Wistar	Male and female	Inhalation	99.8	LC_{50} > 5.7 mg/l	Decker (2001)

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

these results, the acute oral median lethal dose (LD_{50}) was estimated to be greater than 2000 mg/kg bw (Arcelin, 2000).

A group of five male and five female young adult Wistar rats was treated with penthiopyrad dispersed in polyethylene glycol 300 (PEG300) 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 (treatment volume 4 ml/kg bw). The animals were observed for 14 days post-treatment and then subjected to necropsy and postmortem examination.

No deaths or systemic clinical signs of an adverse reaction to treatment occurred. There were no local signs of an effect of treatment at the application site, and there were no macroscopic findings at necropsy in any animal. Two females showed small weight losses (0.6% and 5.3%) from day 1 to day 8, but with subsequent recovery. The weight gain of other animals was within the expected range. Based on these results, the acute dermal LD_{50} was estimated to be greater than 2000 mg/kg bw (Arcelin, 2001).

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 penthiopyrad at an analytically determined mean concentration of 5.669 mg/l, with mass median aerodynamic diameter (MMAD) ± geometric standard deviation (GSD) of 2.81 ± 3.01 (measurement 1) and 2.60 ± 2.91 (measurement 2). 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, and no clinical signs of an adverse reaction to treatment were evident during exposure. All animals showed hunched posture and a moderate decrease in spontaneous activity approximately 1 hour after exposure, and 9 of 10 animals also showed ruffled fur. Clinical signs persisted for up to 2 days. There was transient, marginal weight loss in 5 of 10 animals during the 3 days following exposure, but thereafter all animals showed normal weight gain. 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 5.669 mg/l air (Decker, 2001).

(b) Ocular irritation

In a primary eye irritation study, approximately 0.1 g of penthiopyrad (purity 99.8%) was instilled into the left conjunctival sac of three female New Zealand White rabbits, without subsequent irrigation. A second group of three similarly treated animals had the eyes irrigated with lukewarm water 30 seconds after instillation for 30–60 seconds.

In the non-irrigated group, no corneal or iridial lesions occurred in the animals at any observation interval. All three animals showed grade 1 or grade 2 conjunctival redness and conjunctival chemosis for up to 24 hours after instillation. All animals were free of ocular reactions at 48 and

Table 8. Irritation and skin sensitization potential of penthiopyrad

Species	Strain	Sex	End-point (method)	Purity (%)	Result	Reference
Rabbit	New Zealand White	Female	Skin irritation	99.8	Not irritating	Ueda (2001b)
Rabbit	New Zealand White	Female	Eye irritation	99.8	Minimally irritating	Ueda (2001a)
Guinea-pig	Hartley	Female	Skin sensitization (Magnusson & Kligman)	99.8	Not sensitizing	Ueda (2001c)

72 hours after instillation. Three animals had discharge with moistening of the lids at 1 hour only. In the irrigated group, no corneal or iridial lesions occurred in the animals at any observation interval. All three animals showed grade 1 or grade 2 conjunctival redness and conjunctival chemosis at 1 hour. One animal also showed redness at 24 hours. All animals were free of ocular reactions at 48 and 72 hours after instillation. One animal had discharge with moistening of the lids at 1 hour only. Penthiopyrad is minimally irritating to rabbit eyes (Ueda, 2001a).

(c) Dermal irritation

In a primary skin irritation study, 0.5 g of penthiopyrad (purity 99.8%) moistened with 0.5 ml of distilled water was applied to the shorn skin of three young adult female New Zealand White rabbits. The treated skin area was 2.54 cm × 2.54 cm, and exposure lasted for 4 hours. A second shaved and occluded site served as a control.

No dermal responses were observed in any rabbits during the observation period, and there were no signs of toxicity or ill-health. Penthiopyrad was not irritating to rabbit skin (Ueda, 2001b).

(d) Dermal sensitization

The skin sensitization potential of penthiopyrad (purity 99.8%) was investigated in the maximization test in female Hartley guinea-pigs (20 test and 10 negative control). Two concurrent groups of 10 test and 5 negative control animals were used as a dinitrochlorobenzene (DNCB) positive control. Concentrations of 5%, 50% and 50% penthiopyrad and 0.1%, 1% and 0.5% DNCB 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 penthiopyrad applied were determined in preliminary irritation studies.

None of the 20 test or 10 negative control animals showed a reaction to the challenge application at 24 and 48 hours after patch removal, and all scores were zero. All DNCB-treated animals showed a challenge score of 3 (intense redness and swelling), and the sensitization incidence was 100%. Penthiopyrad was not sensitizing to skin in the maximization test in guinea-pigs (Ueda, 2001c).

The above ocular and dermal irritation and skin sensitization studies are summarized in Table 8.

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

In a 28-day range-finding study, groups of six CD-1 mice of each sex per dose received penthiopyrad (99.8% purity) in the diet at a target dose level of 0, 30, 100, 300 or 1000 mg/kg bw per day.

Table 9. Group mean values for selected haematological and plasma chemistry parameters in mice

Dose (mg/kg bw per day)	Group mean value ± standard deviation					
	Haemoglobin (g/dl)	Red blood cells (10^6/mm^3)	Albumin (g/dl)	Globulin (g/dl)	Albumin to globulin ratio	Triglycerides (mg/dl)
Males						
0	13.0 ± 0.3	7.99 ± 0.26	1.93 ± 0.10	2.55 ± 0.04	0.76 ± 0.04	106 ± 27
30	13.6 ± 0.6	8.23 ± 0.31	1.90 ± 0.13	2.59 ± 0.13	0.74 ± 0.06	153 ± 31
100	12.9 ± 0.4	8.04 ± 0.35	1.89 ± 0.13	2.52 ± 0.16	0.75 ± 0.06	173 ± 24*
300	13.5 ± 0.5	8.12 ± 0.49	1.79 ± 0.14	2.66 ± 0.09	0.67 ± 0.03*	189 ± 23**
1000	13.2 ± 0.7	7.91 ± 0.49	1.72 ± 0.07*	2.79 ± 0.20	0.62 ± 0.05*	190 ± 62**
Females						
0	14.0 ± 0.8	8.26 ± 0.43	2.01 ± 0.09	2.18 ± 0.12	0.92 ± 0.04	134 ± 85
30	13.3 ± 0.5	7.94 ± 0.27	1.98 ± 0.11	2.19 ± 0.08	0.91 ± 0.05	134 ± 58
100	13.8 ± 0.4	8.28 ± 0.57	1.97 ± 0.07	2.27 ± 0.08	0.87 ± 0.05	121 ± 49
300	13.4 ± 0.7	7.79 ± 0.21	1.99 ± 0.12	2.26 ± 0.05	0.88 ± 0.03	161 ± 51
1000	12.8 ± 0.8*	7.56 ± 0.44*	1.84 ± 0.10	2.33 ± 0.09*	0.79 ± 0.05**	119 ± 75

From Nakashima (2001a)
* $P < 0.05$; ** $P < 0.01$

The achieved doses were 0, 30.0, 100, 304 and 1010 mg/kg bw per day in males and 0, 31.4, 104, 330 and 1088 mg/kg bw per day in females. 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 brain, heart, liver, kidneys, lungs and spleen 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 unaffected by treatment at all dose levels. Haemoglobin concentrations and red blood cell counts were slightly reduced in females at 1000 mg/kg bw per day, but all other haematological parameters were unaffected by treatment. Both sexes at 1000 mg/kg bw per day and males at 300 mg/kg bw per day showed reduced plasma albumin and increased globulin concentrations, resulting in reduced albumin to globulin ratio, but total protein values were not affected (Table 9). The group mean plasma triglyceride concentration in males, but not females, at 100, 300 and 1000 mg/kg bw per day was significantly increased. There were no treatment-related effects at any dose level on macroscopic pathology at necropsy, but relative and absolute liver weights showed a dose-related increase in both sexes treated at 300 or 1000 mg/kg bw per day. Treatment-related histopathological alterations were confined to slight, diffuse hepatocellular hypertrophy in both sexes treated at 1000 mg/kg bw per day, but not at lower dose levels.

The no-observed-adverse-effect levels (NOAELs) were 100 and 300 mg/kg bw per day in males and females, respectively, based on the occurrence of decreased albumin concentration and albumin to globulin ratio in males at 300 mg/kg bw per day and in females at 1000 mg/kg bw per day (Nakashima, 2001a). Additionally, there were minor haematological alterations and hepatocellular hypertrophy at 1000 mg/kg bw per day.

In a 90-day oral toxicity study, groups of 10 CD-1 mice of each sex per dose received penthiopyrad (purity 99.8%) in the diet at a target dose level of 0, 30, 100, 300 or 1000 mg/kg bw

per day for 13 weeks. Clinical signs were recorded at least daily, and a detailed physical examination was performed weekly. Ophthalmoscopic examinations were performed pre-dosing and in animals treated at 0 and 1000 mg/kg bw per day in week 13. Body weights and feed consumption were recorded weekly, and haematology and plasma clinical chemistry 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 the control and high-dose groups and liver, kidneys, lungs, thyroid and gross lesions from all animals were processed and examined by light microscopy. Data were analysed statistically where appropriate.

There were no deaths, no treatment-related clinical signs, no effect on feed consumption and no adverse effect on body weight gain at any dose level (Table 10). Mild haematological perturbations occurred in both sexes at 1000 mg/kg bw per day, and males at this dose level showed reduced albumin to globulin ratio and increased plasma urea nitrogen concentration. The latter was evident in males at 300 mg/kg bw per day, but there were no correlating histopathological alterations in the kidneys at 1000 mg/kg bw per day (Table 11). Relative liver weights were increased in both sexes at 300 and 1000 mg/kg bw per day, and absolute and relative thyroid weights were increased at 1000 mg/kg bw per day in males (Tables 12 and 13). Treatment-related histopathological alterations were confined to the liver and thyroid gland. Slight to moderate diffuse hepatocellular hypertrophy and slight thyroid follicular cell hypertrophy occurred in both sexes at 1000 mg/kg bw per day, but not at lower dose levels. Treatment-related histopathological alterations were confined to the liver and thyroid gland. Slight to moderate diffuse hepatocellular hypertrophy occurred in five males and five females at 1000 mg/kg bw per day, but not at lower dose levels or in the controls of either sex. Slight thyroid follicular cell hypertrophy occurred in eight males and six females at 1000 mg/kg bw per day, but not at lower dose levels or in the controls of either sex.

The NOAEL was 100 mg/kg bw per day, based on the occurrence of increased blood urea nitrogen concentration in males and increased relative liver weight at 300 mg/kg bw per day (Nakashima, 2002). Mild haematological perturbations, reduced albumin to globulin ratio and histopathological alterations in the liver and thyroid were seen at 1000 mg/kg bw per day.

Rats

In a 4-week range-finding study, groups of five Wistar rats of each sex per dose received penthiopyrad (purity > 97%) in the diet at a target dose level of 0, 25, 65, 160, 400 or 1000 mg/kg bw per day. Clinical signs were recorded at least daily, a detailed physical examination was performed weekly and a functional observational battery (FOB) 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.

There were no deaths and no adverse clinical signs at any dose level, other than soft faeces at 1000 mg/kg bw per day. Feed consumption was not affected by treatment at any dose level. Similarly, in week 4, there were no effects of treatment at any dose level on the FOB, locomotor activity or grip strength. No treatment-related effects were observed at 160 mg/kg bw per day or lower. At 400 mg/kg bw per day and above, there was a slight prolongation of activated partial thromboplastin time (APTT) in males, as well as increases in plasma total cholesterol and phospholipid levels, gamma-glutamyltransferase (GGT) activity and increased absolute (females only) and relative liver weights in both sexes (Table 14). Body weight and body weight gain were significantly reduced in both sexes at 1000 mg/kg bw per day, but not at lower dose levels. The overall group mean body weight gain at 1000 mg/kg bw per day was reduced by 46.7% and 29.7% in males and females, respectively. Increased triglycerides were observed in females at 1000 mg/kg bw per day. Total bilirubin concentrations were

Table 10. Group mean body weight in mice at selected intervals

Study week	Group mean body weight (g) ± standard deviation									
	Males					Females				
	Dose (mg/kg bw per day)									
	0	30	100	300	1000	0	30	100	300	1000
0	33.0 ± 2.1	33.1 ± 2.0	32.9 ± 2.6	32.9 ± 1.7	32.7 ± 2.0	25.3 ± 1.6	25.2 ± 1.9	25.6 ± 1.6	25.2 ± 1.7	25.3 ± 1.2
1	36.5 ± 2.2	36.5 ± 2.5	36.3 ± 3.3	36.2 ± 1.9	35.9 ± 1.7	26.7 ± 1.5	26.9 ± 2.3	27.4 ± 2.4	27.0 ± 1.5	27.5 ± 1.7
2	38.4 ± 2.6	38.8 ± 2.7	38.3 ± 3.3	37.9 ± 1.8	37.4 ± 1.8	27.8 ± 2.3	27.8 ± 2.0	28.9 ± 2.4	28.6 ± 1.9	28.6 ± 1.7
4	41.4 ± 3.0	42.0 ± 3.5	40.5 ± 4.4	40.1 ± 1.8	39.3 ± 2.0	29.4 ± 2.0	29.4 ± 2.7	31.1 ± 2.4	31.1 ± 1.5	30.1 ± 0.9
8	46.4 ± 4.6	46.8 ± 4.6	44.4 ± 5.6	43.5 ± 2.2	42.7 ± 2.5	32.4 ± 2.3	32.2 ± 3.5	33.5 ± 4.1	33.7 ± 1.9	32.2 ± 2.6
13	51.0 ± 5.5	52.2 ± 6.1	48.1 ± 6.0	47.5 ± 2.9	46.1 ± 3.2	34.3 ± 2.6	35.4 ± 5.3	38.1 ± 4.1	37.2 ± 2.6	34.4 ± 4.6

From Nakashima (2002). Data from report tables 5 and 6, pp. 38 and 39.

Table 11. Group mean values for selected haematological and plasma chemistry parameters in mice

Dose (mg/kg bw per day)	Group mean value ± standard deviation					
	Haemoglobin (g/dl)	Red blood cells (10^6/mm^3)	Albumin (g/dl)	Globulin (g/dl)	Albumin to globulin ratio	Blood urea nitrogen (mg/dl)
Males						
0	13.3 ± 0.07	8.20 ± 0.47	2.00 ± 0.25	2.63 ± 0.19	0.77 ± 0.10	31.5 ± 6.8
30	13.3 ± 0.06	8.22 ± 0.39	2.06 ± 0.19	2.59 ± 0.17	0.80 ± 0.07	31.2 ± 3.7
100	13.2 ± 0.07	8.06 ± 0.40	1.85 ± 0.18	2.65 ± 0.18	0.70 ± 0.10	39.6 ± 9.9
300	13.0 ± 0.06	7.94 ± 0.43	1.94 ± 0.14	2.70 ± 0.09	0.72 ± 0.06	42.7 ± 8.8**
1000	12.8 ± 0.05	7.66 ± 0.36*	1.85 ± 0.20	2.80 ± 0.09	0.66 ± 0.08*	40.3 ± 6.6*
Females						
0	13.6 ± 0.05	8.11 ± 0.41	2.07 ± 0.19	2.36 ± 0.14	0.88 ± 0.10	27.9 ± 5.9
30	13.9 ± 0.09	8.29 ± 0.69	2.20 ± 0.09	2.31 ± 0.11	0.95 ± 0.05	27.8 ± 6.1
100	13.6 ± 0.04	8.05 ± 0.23	2.11 ± 0.19	2.30 ± 0.15	0.92 ± 0.10	27.5 ± 6.0
300	13.1 ± 0.05	7.76 ± 0.37	2.03 ± 0.15	2.33 ± 0.11	0.87 ± 0.08	30.9 ± 5.8
1000	12.6 ± 0.08**	7.39 ± 0.40**	1.93 ± 0.35	2.55 ± 0.49	0.79 ± 0.20	27.8 ± 5.3

From Nakashima (2002). Data from report tables 15-1, 17-11 and 18-1, pp. 48, 52 and 54.
* $P < 0.05$; ** $P < 0.01$

Table 12. Group mean body weights and selected absolute and relative organ weights at 13 weeks in male mice

Dose (mg/kg bw per day)	Body weights (g)	Group mean organ weights ± standard deviation			
		Liver		Thyroid	
		g	% of body weight	g	% of body weight
0	51.0 ± 5.5	2.53 ± 0.21	4.97 ± 0.32	4.8 ± 1.1	0.010 ± 0.003
30	52.2 ± 6.1	2.83 ± 0.68	5.38 ± 0.72	5.3 ± 1.1	0.010 ± 0.001
100	48.1 ± 6.0	2.60 ± 0.48	5.37 ± 0.43	5.4 ± 1.6	0.011 ± 0.003
300	47.5 ± 2.9	2.67 ± 0.26	5.62 ± 0.53*	5.3 ± 1.1	0.011 ± 0.003
1000	46.1 ± 3.2	2.83 ± 0.22	6.15 ± 0.26**	6.9 ± 1.1**	0.015 ± 0.002**

From Nakashima (2002). Data from report tables 19-1 and 19-3, pp. 56 and 58.
* $P < 0.05$; ** $P < 0.01$

Table 13. Group mean body weights and selected absolute and relative organ weights at 13 weeks in female mice

Dose (mg/kg bw per day)	Body weights (g)	Group mean organ weights ± standard deviation			
		Liver		Thyroid	
		g	% of body weight	g	% of body weight
0	34.3 ± 2.6	1.71 ± 0.21	4.99 ± 0.38	4.6 ± 1.1	0.013 ± 0.003
30	35.4 ± 5.3	1.80 ± 0.34	5.06 ± 0.51	4.2 ± 1.2	0.012 ± 0.003
100	38.1 ± 4.1	1.99 ± 0.20	5.23 ± 0.43	5.2 ± 1.9	0.014 ± 0.005
300	37.2 ± 2.6	2.07 ± 0.23*	5.55 ± 0.39*	4.7 ± 0.6	0.013 ± 0.002
1000	34.4 ± 4.6	1.99 ± 0.35	5.78 ± 0.50**	5.4 ± 1.1	0.016 ± 0.003

From Nakashima (2002). Data from report tables 20-1 and 20-3, pp. 60 and 62.
* $P < 0.05$; ** $P < 0.01$

Table 14. Selected group mean plasma clinical chemistry data in rats: week 4

Dose (mg/kg bw per day)	Group mean value					
	Total cholesterol (mmol/l)	Triglycerides (mmol/l)	Phospholipid (mmol/l)	GGT (nkat/l)	Total bilirubin (µmol/l)	Creatine kinase (µkat/l)
Males						
0	1.51	0.50	1.46	15.07	1.57	3.68
25	1.75	0.28	1.48	16.74	1.24	4.17
65	1.71	0.36	1.52	14.54	1.14*	4.19
160	1.62	0.49	1.51	18.57	1.24	2.93
400	2.22**	0.60	1.90*	36.01[a]	1.14*	2.98
1000	2.92**	0.55	2.54**	331.5**[a]	1.11**	6.86
Females						
0	0.93	0.24	1.01	5.27	1.54	2.90
25	0.92	0.18[a]	1.02	8.67	1.21	2.87
65	1.34	0.19[a]	1.31	13.57[a]	1.06	2.44
160	1.28	0.21	1.32	15.00[a]	0.86*	2.67
400	1.93**	0.29	1.71**	41.21[a]	0.82*	2.73
1000	2.35**	0.49**[a]	2.15**	665.9**[a]	0.80*	10.62*[b]

From Hamann (2001); Hamann, Knuppe & Weber (2001)
* $P < 0.05$; ** $P < 0.01$ (Dunnett's test)
[a] $P < 0.05$ (Kruskal-Wallis/rank sum test).
[b] Not significant ($P < 0.05$) (Kruskal-Wallis/rank sum test).

Table 15. Incidences of selected liver alterations in rats

Liver lesion	Grade[a]	Incidence in group											
		Dose (mg/kg bw per day)											
		0		25		65		160		400		1000	
		M	F	M	F	M	F	M	F	M	F	M	F
Number examined		*5*	*5*	*5*	*5*	*5*	*5*	*5*	*5*	*5*	*5*	*5*	*5*
Kupffer cell proliferation	1	0	0	0	0	0	0	0	0	0	0	4	3
	2	0	0	0	0	0	0	0	0	0	0	1	0
Fatty change	1	2	5	2	5	3	4	0	3	1	3	0	0
	2	0	0	0	0	0	0	0	0	0	0	2	3
	3	0	0	0	0	0	0	0	0	0	0	3	2
Hypertrophy	1	0	0	0	0	0	0	0	0	0	0	1	1
	2	0	0	0	0	0	0	0	0	0	0	1	0

From Hamann (2001); Hamann, Knuppe & Weber (2001)
F, female; M, male
[a] Grade 1: Minimal / very few / very small; Grade 2: Slight / few / small; Grade 3: Moderate / moderate number / moderate size.

reduced at and above 65 mg/kg bw per day, but this alteration, in isolation, was not considered an adverse effect. There were no treatment-related effects at any dose level on macroscopic pathology at necropsy, but relative and/or absolute liver weights showed a dose-related increase in both sexes treated at 400 or 1000 mg/kg bw per day. Treatment-related histopathological alterations were slight to moderate hepatic microvesicular or macrovesicular fatty change mainly in the peripherolobular region in all animals at 1000 mg/kg bw per day. The fatty change was accompanied by slight Kupffer cell proliferation in most animals and by hepatic hypertrophy in a few animals (Table 15).

The NOAEL was 160 mg/kg bw per day, based on the occurrence of a dose-related altered serum clinical chemistry at 400 mg/kg bw per day. Reduced body weight, reduced body weight gain, altered serum clinical chemistry, increased liver weight and histopathological alterations in the liver (microvesicular/macrovesicular fatty change) were observed at 1000 mg/kg bw per day (Hamann, 2001; Hamann, Knuppe & Weber, 2001).

In a 13-week toxicity study in rats, groups of 10 Wistar rats of each sex per dose received penthiopyrad (purity 99.8%) in the diet at a target dose level of 0, 40, 100, 250 or 625 mg/kg bw per day. Two further groups treated at 0 or 625 mg/kg bw per day for 13 weeks were left untreated for a further 4 weeks. Clinical signs were recorded at least daily, a detailed physical examination was performed weekly and FOB, grip strength and motor activity assessments were performed in week 13. Ophthalmoscopic examinations were performed pre-dosing and in animals treated at 0 and 625 mg/kg bw per day in weeks 13 and 17. Body weights and feed consumption were recorded weekly, and haematology, plasma clinical chemistry and urine analysis were performed in weeks 13 and 17 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 was a single, treatment-related male death at 625 mg/kg bw per day and no adverse clinical signs at any dose level, other than soft faeces at 625 mg/kg bw per day. In week 13, there were no effects of treatment at any dose level on the ocular architecture, FOB or grip strength assessments, but locomotor activity at 625 mg/kg bw per day showed a treatment-related reduction. Body weight gain was significantly reduced in males at 625 mg/kg bw per day, but feed consumption was

not affected by treatment at any dose level. Minor and largely reversible changes in the haematological profile occurred at 625 mg/kg bw per day, comprising reduced haemoglobin concentration, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration and prolonged prothrombin time and/or APTT and increased red blood cell count and a shift to low-fluorescence reticulocytes in females. Minimal changes in some of these parameters also occurred in males at 100 mg/kg bw per day and in both sexes at 250 mg/kg bw per day. Treatment-related effects on the plasma chemistry profile occurred at dose levels of 100 mg/kg bw per day and above. Increased total cholesterol, triglyceride and phospholipid concentrations occurred at 625 mg/kg bw per day, with the effect on some parameters extending to one or both sexes at 250 mg/kg bw per day and to males at 100 mg/kg bw per day. Total bilirubin concentration was reduced in both sexes at 250 and 625 mg/kg bw per day, and alkaline phosphatase and GGT activities were increased in both sexes at 625 mg/kg bw per day. All effects at 625 mg/kg bw per day were reversible. There were no treatment-related effects on the chemical or cellular constituents of urine at any dose level. Treatment-related macroscopic findings at necropsy were confined to the liver of males at 250 and 625 mg/kg bw per day, in which accentuated lobular pattern and thickened or enlarged liver occurred. Absolute and/or relative liver weights showed a dose-related increase in both sexes at 100, 250 and 625 mg/kg bw per day. Reduced absolute and relative spleen weights occurred in both sexes at 625 mg/kg bw per day, and increased ovary weights at 625 mg/kg bw per day. Relative liver weights showed some recovery, but remained slightly elevated at the end of the recovery period.

Treatment-related histopathological alterations occurred in the liver at all dose levels of penthiopyrad. Macrovesicular fatty change, hepatocellular degeneration and Kupffer cell proliferation occurred at dose levels of 100 mg/kg bw per day and above, and hepatocellular hypertrophy was evident in some or all animals at all dose levels of penthiopyrad; at 40 mg/kg bw per day, however, in the absence of the other hepatic alterations, the hypertrophic response alone was considered not to be an adverse reaction to treatment. At the end of the recovery period, hepatocellular hypertrophy remained, but at reduced severity, and the other histopathological alterations had largely regressed.

The NOAEL was 40 mg/kg bw per day, based on the occurrence of increased liver weights along with histopathological alterations in the liver (hepatocellular degeneration, Kupffer cell proliferation) and haematological and clinical chemistry perturbations at and above 100 mg/kg bw per day. Reduced body weight and body weight gain, haematological and clinical chemistry alterations, and more liver histopathology were seen at and above 250 mg/kg bw per day. At 625 mg/kg bw per day, decreased motor activity and mortality were observed (Rosner, Biedermann & Weber, 2005, 2007).

Dogs

In a 4-week range-finding study, groups of one male and one female Beagle dog per dose received penthiopyrad (purity > 99%) in the diet at a concentration of 0, 1000, 3000, 10 000 or 32 000 ppm (equal to dose levels of 0, 27.1, 79.6, 269 and 920 mg/kg bw per day in males and 0, 29.1, 94.1, 316 and 982 mg/kg bw per day in females). The animals were subjected weekly to a detailed clinical examination and body weight and feed consumption recording. Ophthalmological examinations and urinalysis were performed on all animals pre-dosing and in week 4. Blood samples for haematology and plasma clinical chemistry were withdrawn from all animals pre-dosing and in weeks 2 and 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. Brain, pituitary, thyroids with parathyroids, heart, liver with gall bladder, stomach, kidneys, spleen, lungs and gross lesions from all animals were examined by light microscopy.

There were no deaths at any dose level, no treatment-related clinical signs and no effects on feed consumption, ocular architecture, urinalysis or gross lesions at necropsy. Body weight gain was depressed by treatment at 32 000 ppm but not at lower dose levels. Mild, transient decreases in haematocrit, haemoglobin concentration and red blood cell count occurred in the male dog at

32 000 ppm in week 2. Plasma alkaline phosphatase activity and total cholesterol were increased in both sexes at 32 000 ppm and in the female dog at 10 000 ppm at 2 and 4 weeks, but not at lower dose levels. Plasma aspartate aminotransferase and alanine aminotransferase activities were also slightly elevated at week 4 in the male dog at 32 000 ppm. Increased liver weight associated with slight, diffuse hepatocellular hypertrophy occurred in both sexes at 32 000 ppm, in the female dog at 10 000 ppm and in the male dog at 3000 ppm, but these effects were not apparent in the male dog at 10 000 ppm. Treatment of both sexes at and above 3000 ppm was associated with slight mucosal oedema in the propria lamina of the gall bladder.

No NOAEL was established in this study, which was designed only to explore appropriate high doses for further study (Nakashima, 2001b).

In a subchronic oral toxicity study, four Beagle dogs of each sex per dose were administered penthiopyrad (purity 99.1%) in the diet for 13 weeks at a concentration of 0, 300, 3000 or 30 000 ppm (equal to dose levels of 0, 8.0, 76.7 and 811 mg/kg bw per day in males and 0, 8.2, 80.9 and 864 mg/kg bw per day in females). The animals were subjected weekly to a detailed clinical examination and body weight and feed consumption recording. Ophthalmological examinations were performed on all animals pre-dosing and in week 13. Blood samples for haematology and plasma clinical chemistry were withdrawn, and urine samples were collected, from all animals pre-dosing and in weeks 7 and 13. The animals were sacrificed after 91 days' 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, no treatment-related clinical signs and no effects on ocular architecture, urinalysis and gross lesions at necropsy 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. Overall body weight gain in both sexes at 30 000 ppm showed a treatment-related decrease (about 44%), resulting in group mean body weights in week 13 that were 9.3% and 7.1% lower than control values in males and females, respectively (Table 16). There were no treatment-related adverse changes in the haematological profile. Plasma alkaline phosphatase activity and total bilirubin concentration were increased in both sexes, and GGT activity was increased in females at 30 000 ppm, but not at lower dose levels (generally at weeks 7 and 13). In addition, plasma albumin concentration and the albumin to globulin ratio were reduced and total cholesterol and triglyceride concentrations tended to be increased in this group, and these alterations were considered to reflect hepatic dysfunction (Table 17). There were no treatment-related gross pathological lesions at necropsy at any dose level, and effects on organ weights were confined to increased absolute and relative liver weights in both sexes (absolute/relative 36%/24% in males and 50%/34% in females) at 30 000 ppm and increased thyroid weights in females (absolute/relative 34%/42%) at 30 000 ppm (Table 18). Treatment-related histopathological alterations were confined to the liver and gall bladder of both sexes at 30 000 ppm and the adrenal glands of males at 30 000 ppm. Diffuse hepatocellular hypertrophy (slight to moderate, all animals), cholecystitis in the gall bladder (moderate; three males and all females) and adrenal cortical cell hypertrophy (moderate; all males) were the defining lesions (Table 19). There were no other treatment-related histopathological alterations in other tissues, including the thyroid gland of females, in which increased weight was evident.

The NOAEL was 3000 ppm (equal to 76.7 and 80.9 mg/kg bw per day in males and females, respectively) (Nakashima, 2001c), based on the occurrence of reduced weight gain, clinical indicators of hepatic dysfunction and histopathological alterations in the liver, gall bladder and adrenal glands at 30 000 ppm (equal to 811 and 864 mg/kg bw per day in males and females, respectively).

In a 1-year oral toxicity study, groups of four Beagle dogs of each sex per dose were treated for 52 weeks with penthiopyrad (purity 98.8%) incorporated into the diet at concentrations of 0, 310,

Table 16. Selected group mean body weights in dogs

Dietary concentration (ppm)	Group mean body weight (kg) ± standard deviation					
	Week					
	0	1	2	4	8	13
Males						
0	8.5 ± 0.2	8.8 ± 0.3	9.0 ± 0.3	9.5 ± 0.2	10.2 ± 0.2	10.8 ± 0.2
300	8.4 ± 0.4	8.7 ± 0.4	8.8 ± 0.5	9.1 ± 0.5	9.5 ± 0.6	9.8 ± 0.7
3000	8.5 ± 0.4	8.7 ± 0.4	8.9 ± 0.4	9.4 ± 0.4	10.0 ± 0.6	10.6 ± 0.7
30 000	8.5 ± 0.3	8.5 ± 0.3	8.6 ± 0.3	8.9 ± 0.3	9.4 ± 0.5	9.8 ± 0.6
Females						
0	8.2 ± 0.3	8.4 ± 0.4	8.5 ± 0.5	8.8 ± 0.5	9.2 ± 0.7	9.8 ± 0.8
300	8.1 ± 0.5	8.4 ± 0.5	8.5 ± 0.5	8.8 ± 0.4	9.3 ± 0.5	9.9 ± 0.6
3000	8.2 ± 0.3	8.4 ± 0.3	8.6 ± 0.3	9.0 ± 0.4	9.5 ± 0.4	9.9 ± 0.5
30 000	8.2 ± 0.5	8.1 ± 0.5	8.2 ± 0.5	8.4 ± 0.7	8.8 ± 0.6	9.1 ± 0.5

From Nakashima (2001c). Data extracted from report tables 7 and 8, pp. 52–53.

Table 17. Selected haematological and plasma chemistry values in dogs

Dietary concentration (ppm)	Week	Group mean value ± standard deviation						
		APTT (s)	GGT (U/l)	Albumin (g/dl)	Albumin to globulin ratio	Triglycerides (mg/dl)	Alkaline phosphatase (U/l)	Total bilirubin (mg/dl)
Males								
0	0	13.6 ± 0.4	4 ± 1	2.90 ± 0.16	1.06 ± 0.12	37 ± 4	116 ± 14	0.16 ± 0.01
	7	13.9 ± 0.7	4 ± 1	2.98 ± 0.15	1.14 ± 0.9	36 ± 6	90 ± 13	0.12 ± 0.04
	13	13.3 ± 0.5	4 ± 1	3.13 ± 0.23	1.10 ± 0.11	37 ± 9	70 ± 9	0.14 ± 0.02
300	0	13.7 ± 1.9	4 ± 1	2.92 ± 0.12	1.00 ± 0.18	43 ± 11	133 ± 8	0.16 ± 0.01
	7	14.1 ± 1.4	4 ± 1	3.21 ± 0.12	1.05 ± 0.17	48 ± 11	117 ± 20	0.13 ± 0.02
	13	13.7 ± 1.1	4 ± 1	3.18 ± 0.13	1.04 ± 0.15	49 ± 9	102 ± 17	0.16 ± 0.01
3000	0	13.7 ± 0.8	4 ± 1	2.93 ± 0.11	1.10 ± 0.12	34 ± 7	119 ± 13	0.16 ± 0.01
	7	12.9 ± 0.4	4 ± 1	3.03 ± 0.14	1.10 ± 0.10	44 ± 13	90 ± 16	0.15 ± 0.02
	13	13.1 ± 0.6	5 ± 1	3.17 ± 0.21	1.07 ± 0.14	42 ± 14	79 ± 15	0.14 ± 0.02
30 000	0	13.5 ± 0.2	4 ± 1	2.98 ± 0.19	1.09 ± 0.8	41 ± 9	128 ± 23	0.16 ± 0.01
	7	12.1 ± 0.7*	5 ± 0	2.64 ± 0.25*	0.81 ± 0.15*	58 ± 14	237 ± 76*	0.19 ± 0.01**
	13	12.3 ± 0.5	5 ± 1	2.55 ± 0.37*	0.72 ± 0.20*	60 ± 11	299 ± 81**	0.21 ± 0.01**
Females								
0	0	14.0 ± 0.7	3 ± 0	2.83 ± 0.13	1.12 ± 0.11	46 ± 8	116 ± 22	0.09 ± 0.01
	7	13.9 ± 10.5	3 ± 1	3.06 ± 0.08	1.07 ± 0.18	51 ± 9	92 ± 17	0.13 ± 0.03
	13	14.2 ± 0.7	3 ± 0	3.06 ± 0.12	0.98 ± 0.10	43 ± 5	93 ± 16	0.17 ± 0.02
300	0	13.7 ± 1.0	4 ± 01	2.96 ± 0.08	1.14 ± 0.14	40 ± 5	133 ± 24	0.09 ± 0.01
	7	13.5 ± 0.4	4 ± 1	3.15 ± 0.12	1.13 ± 0.28	48 ± 10	93 ± 11	0.14 ± 0.01
	13	13.9 ± 0.8	4 ± 1	3.31 ± 0.12	1.13 ± 0.24	41 ± 7	82 ± 11	0.18 ± 0.02
3000	0	13.7 ± 0.3	4 ± 1	2.90 ± 0.6	1.22 ± 0.06	35 ± 6	127 ± 15	0.10 ± 0.01
	7	12.5 ± 0.5**	3 ± 11	3.19 ± 0.22	1.19 ± 0.18	41 ± 3	104 ± 16	0.15 ± 0.02
	13	13.4 ± 0.5	4 ± 1	3.05 ± 0.25	0.99 ± 0.15	40 ± 5	95 ± 25	0.16 ± 0.02

Table 17 (continued)

Dietary concentration (ppm)	Week	Group mean value ± standard deviation						
		APTT (s)	GGT (U/l)	Albumin (g/dl)	Albumin to globulin ratio	Triglycerides (mg/dl)	Alkaline phosphatase (U/l)	Total bilirubin (mg/dl)
30 000	0	14.0 ± 0.8	4 ± 1	2.84 ± 0.08	1.08 ± 0.05	42 ± 6	105 ± 20	0.10 ± 0.02
	7	12.2 ± 0.7**	5 ± 1**	2.59 ± 0.40*	0.70 ± 0.14	62 ± 4	274 ± 71*	0.20 ± 0.02**
	13	12.4 ± 1.2	5 ± 1*	2.67 ± 0.18*	0.70 ± 0.08	59 ± 4**	338 ± 155	0.23 ± 0.02**

From Nakashima (2001c). Data extracted from report tables 17-3, 18-3, 19-1, 19-3, 19-4, 20-1, 20-3 and 20-4, pp. 74, 79, 82, 84, 85, 87, 89 and 90, respectively.
APTT, activated partial thromboplastin time; GGT, gamma-glutamyltransferase; U, unit; * $P < 0.05$; ** $P < 0.01$

Table 18. Selected individual absolute and relative organ weights in dogs

Dietary concentration (ppm)	Individual values ± standard deviation			
	Liver		Thyroid	
	g	% of body weight	mg	% of body weight
Males				
0	251 ± 24	2.33 ± 0.18	683 ± 136	0.0063 ± 0.0012
300	262 ± 32	2.69 ± 0.54	902 ± 187	0.0092 ± 0.0016
3000	287 ± 25	2.72 ± 0.29	946 ± 97	0.0090 ± 0.0015
30 000	341 ± 14**	3.50 ± 0.25**	871 ± 376	0.0090 ± 0.0043
Females				
0	241 ± 25	2.47 ± 0.28	701 ± 91	0.0073 ± 0.0015
300	235 ± 9	2.39 ± 0.09	870 ± 107	0.0089 ± 0.0014
3000	244 ± 33	2.48 ± 0.47	700 ± 72	0.0071 ± 0.0009
30 000	301 ± 31*	3.30 ± 0.25**	941 ± 172*	0.0104 ± 0.0019*

From Nakashima (2001c). Data extracted from report tables 22-1 and 22-2, pp. 94–95.
* $P < 0.05$; ** $P < 0.01$

Table 19. Incidences of selected histopathological alterations in dogs

Tissue/lesion	Incidence in group			
	Dietary concentration (ppm)			
	0	300	3000	30 000
Males (n = 4)				
Adrenal cortical cell hypertrophy	0	0	0	4*
Liver diffuse hepatocyte hypertrophy	0	0	0	4*
Gall bladder cholecystitis	0	0	0	3
Females (n = 4)				
Liver diffuse hepatocyte hypertrophy	0	0	0	4*
Gall bladder cholecystitis	0	0	0	4*

From Nakashima (2001c). Data extracted from report tables 25-1, 25-2 and 26-2, pp. 98, 99 and 102.
* $P < 0.05$

2150 or 15 000 ppm (equal to average dose levels of 0, 7.9, 54.4 and 461 mg/kg bw per day in males and 0, 8.1, 56.6 and 445 mg/kg bw per day in females). 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 pre-dosing and in week 52. Blood samples for haematology and plasma clinical chemistry were withdrawn, and urine samples were collected, from all animals pre-dosing 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 were preserved, processed and examined by light microscopy.

There were no deaths at any dose level, and there were no treatment-related clinical signs or effects in the semiquantitative observations made in the home cage and in the open field. Ophthalmological parameters were also unaffected by treatment. Brief episodes of clonic convulsions observed on day 1 at 15 000 ppm at 24 hours post-dosing in two males and one female and on nine other occasions in one of these males during the last 16 weeks were not considered treatment related because no effects were seen in the subchronic study at twice the dose. There was treatment-related initial weight loss followed by reduced weight gain at 15 000 ppm, but not at lower dose levels, leading to body weights that were 22% and 17% lower than those of controls in males and females at week 52, respectively (Table 20). The feed consumption of both sexes at 15 000 ppm was reduced relative to the controls in week 1 (−14% to −15%), followed by a return to normal consumption. Feed consumption at lower dose levels was unaffected by treatment. Treatment-related changes in the haematological profile occurred at 15 000 ppm, but not at lower dose levels. Males showed reduced red blood cell count and haemoglobin concentration (−11% to −14%) and increased numbers of platelets (+60% to +70%) at 13, 26 and 52 weeks; females were not affected. At 15 000 ppm, both sexes showed a decrease (about −20%) in APTT at all sampling intervals during the treatment period (Table 21). Treatment-related changes in the plasma chemistry profile occurred at 15 000 ppm. The plasma activities of alkaline phosphatase (5- to 11-fold greater than controls) and GGT (2.5- to 4.5-fold above controls) and concentrations of total cholesterol (+45% to +51%) and triglycerides (about 2-fold greater than controls) were elevated in males at 15 000 ppm in weeks 13, 26 and 52. The effect on alkaline phosphatase activity was particularly marked and progressed in severity during the treatment period. Females at 15 000 ppm showed similar effects, but triglycerides and cholesterol did not show statistically significant changes. Albumin concentrations were reduced and globulin concentrations were increased at this dose level in both sexes, resulting in reduced albumin to globulin ratios (−25% to −29% below controls). Although the group mean globulin concentration tended to be higher than the control value and the group mean albumin to globulin ratio tended to be lower in females at 2150 ppm, the differences from the control group were due mainly to a single female that showed a relatively high globulin level throughout the treatment period, together with raised alkaline phosphatase activity. These values were not typical of the group and were not observed in the subchronic study at a dose of 3000 ppm. As this female did not show the elevated triglyceride and total cholesterol concentrations seen at 15 000 ppm and showed no histopathological alterations in the liver, the higher alkaline phosphatase activity and lower albumin to globulin ratio alone were not considered adverse effects (Table 22). There were no other treatment-related effects on the plasma chemistry profile, and urinalysis parameters were also unaffected by treatment. There were no unequivocal, treatment-related gross pathological lesions at necropsy at any dose level, although two of four males at 15 000 ppm showed ascites. Effects on organ weights occurred at 15 000 ppm, but not at lower dose levels. Absolute and relative liver and adrenal weights were increased in both sexes at 15 000 ppm (absolute values were +30% to +51% above controls). There were no other treatment-related effects on organ weights at any dose level (Table 23). Treatment-related histopathological alterations occurred in the liver, adrenal glands and gall bladder of both sexes at 15 000 ppm, but not at lower dose levels. The lesions identified were slight diffuse hepatocellular hypertrophy (all animals), slight or moderate

Table 20. Selected group mean body weights in dogs

Dietary concentration (ppm)	Group mean body weight (kg) ± standard deviation						
	Week						
	0	1	4	13	28	40	52
Males							
0	8.0 ± 0.3	8.1 ± 0.4	8.7 ± 0.5	10.0 ± 0.6	10.5 ± 0.9	10.6 ± 1.0	10.6 ± 1.0
310	8.0 ± 0.4	8.2 ± 0.4	8.8 ± 0.5	9.7 ± 0.8	10.0 ± 1.1	10.1 ± 1.0	10.1 ± 1.0
2150	8.0 ± 0.3	8.3 ± 0.4	8.9 ± 0.6	9.7 ± 1.2	10.0 ± 1.9	10.3 ± 1.8	10.3 ± 1.8
15 000	8.0 ± 0.3	7.8 ± 0.5	7.9 ± 0.5	7.9 ± 0.8*	8.0 ± 0.6*	8.3 ± 0.7	8.3 ± 0.9
Females							
0	7.3 ± 0.3	7.6 ± 0.3	8.1 ± 0.3	9.1 ± 0.6	9.7 ± 1.2	10.0 ± 1.3	10.2 ± 1.8
310	7.4 ± 0.3	7.5 ± 0.3	8.0 ± 0.2	9.2 ± 0.3	9.9 ± 0.3	10.2 ± 0.2	10.0 ± 0.2
2150	7.4 ± 0.6	7.6 ± 0.6	8.1 ± 0.6	9.1 ± 0.9	9.8 ± 1.3	10.1 ± 1.4	10.0 ± 1.6
15 000	7.4 ± 0.6	7.3 ± 0.7	7.6 ± 1.0	8.1 ± 1.5	8.4 ± 1.6	8.7 ± 1.5	8.5 ± 1.4

From Nakashima (2006). Data extracted from report tables 7 and 8, pp. 112 and 113.
* $P < 0.05$

Table 21. Selected group mean haematological values in dogs

Dietary concentration (ppm)	Week	Group mean values ± standard deviation			
		Red blood cells (10^6/µl)	Haemoglobin (g/dl)	Platelet count (10^3/µl)	APTT (s)
Males					
0	0	6.68 ± 0.20	14.6 ± 0.9	346 ± 78	14.8 ± 1.5
	13	7.30 ± 0.27	16.0 ± 1.2	330 ± 73	14.7 ± 1.2
	26	7.28 ± 0.19	15.8 ± 0.9	338 ± 68	15.9 ± 1.1
	52	7.76 ± 0.26	17.0 ± 1.0	306 ± 62	14.8 ± 1.3
310	0	6.57 ± 0.09	14.6 ± 0.6	342 ± 58	13.9 ± 0.2
	13	7.10 ± 0.26	15.9 ± 1.2	352 ± 73	14.3 ± 0.4
	26	7.30 ± 0.25	16.1 ± 1.2	378 ± 79	15.7 ± 0.5
	52	7.62 ± 0.48	16.9 ± 1.2	351 ± 87	14.3 ± 0.7
2150	0	6.49 ± 0.50	14.2 ± 1.1	305 ± 126	14.2 ± 0.8
	13	7.01 ± 0.24	15.7 ± 0.4	378 ± 29	13.6 ± 0.7
	26	7.19 ± 0.18	15.8 ± 0.4	389 ± 62	15.5 ± 0.8
	52	7.44 ± 0.26	16.6 ± 0.6	370 ± 39	13.5 ± 0.6
15 000	0	6.64 ± 0.57	14.6 ± 1.4	361 ± 40	14.2 ± 0.9
	13	6.37 ± 0.10**	14.4 ± 0.3	527 ± 110**	12.1 ± 0.5**
	26	6.47 ± 0.33**	14.4 ± 0.7	567 ± 50**	13.5 ± 0.3**
	52	6.64 ± 0.33**	14.9 ± 0.7*	522 ± 53**	12.1 ± 0.3**
Females					
0	0	6.71 ± 0.31	14.8 ± 0.8	361 ± 53	14.4 ± 0.5
	13	7.21 ± 0.45	16.0 ± 1.0	375 ± 56	14.3 ± 0.6
	26	7.03 ± 0.69	15.6 ± 1.6	402 ± 76	13.8 ± 1.3
	52	7.34 ± 0.97	16.6 ± 2.0	389 ± 89	14.5 ± 1.1

Table 21 (continued)

Dietary concentration (ppm)	Week	Group mean values ± standard deviation			
		Red blood cells (10^6/µl)	Haemoglobin (g/dl)	Platelet count (10^3/µl)	APTT (s)
310	0	6.93 ± 0.43	15.5 ± 0.8	316 ± 52	13.9 ± 0.8
	13	7.50 ± 0.33	17.0 ± 0.3	338 ± 43	13.8 ± 0.5
	26	7.27 ± 0.20	16.4 ± 0.3	350 ± 38	13.4 ± 0.7
	52	7.77 ± 0.20	17.5 ± 0.5	320 ± 39	14.3 ± 0.5
2150	0	6.77 ± 0.33	14.6 ± 1.2	344 ± 56	14.5 ± 0.2
	13	7.32 ± 0.88	16.2 ± 2.3	330 ± 37	13.3 ± 0.3
	26	7.01 ± 0.69	15.4 ± 1.5	312 ± 87	12.9 ± 0.8
	52	7.59 ± 0.81	16.8 ± 1.9	312 ± 25	13.5 ± 0.2
15 000	0	6.88 ± 0.29	15.2 ± 1.0	324 ± 51	14.3 ± 0.3
	13	7.40 ± 0.12	16.7 ± 0.3	402 ± 63	11.6 ± 0.7**
	26	7.26 ± 0.34	16.2 ± 1.1	426 ± 55	11.3 ± 0.4**
	52	7.55 ± 0.66	16.9 ± 1.4	411 ± 50	11.6 ± 0.3*

From Nakashima (2006). Data extracted from report tables 17-1, 71-3, 17-4, 18-1, 18-3 and 18-4, pp. 138, 140–141, 145 and 147–148.
APTT, activated partial thromboplastin time; * $P < 0.05$; ** $P < 0.01$

Table 22. Selected group mean plasma chemistry values in dogs

Dietary concentration (ppm)	Week	Group mean values ± standard deviation						
		Alkaline phosphatase (U/l)	GGT (U/l)	Albumin (g/dl)	Globulin (mg/dl)	Albumin to globulin ratio	Triglycerides (mg/dl)	Total cholesterol (mg/dl)
Males								
0	0	458 ± 51	3 ± 1	3.04 ± 0.09	2.31 ± 0.20	1.32 ± 0.08	32 ± 8	151 ± 17
	13	315 ± 23	4 ± 1	3.30 ± 0.14	2.90 ± 0.28	1.15 ± 0.12	22 ± 9	150 ± 14
	26	203 ± 18	4 ± 1	3.18 ± 0.20	3.07 ± 0.34	1.05 ± 0.10	22 ± 8	146 ± 17
	52	209 ± 32	4 ± 1	3.29 ± 0.16	3.17 ± 0.40	1.05 ± 0.14	14 ± 5	148 ± 16
310	0	516 ± 87	3 ± 0	3.21 ± 0.16	2.42 ± 0.26	1.33 ± 0.08	33 ± 12	162 ± 26
	13	351 ± 53	4 ± 1	3.37 ± 0.16	3.10 ± 0.35	1.10 ± 0.09	20 ± 7	157 ± 21
	26	294 ± 139	5 ± 1	3.31 ± 0.15	3.39 ± 0.38	0.98 ± 0.08	18 ± 3	136 ± 23
	52	248 ± 79	5 ± 1	3.40 ± 0.23	3.52 ± 0.40	0.98 ± 0.10	14 ± 4	132 ± 23
2150	0	489 ± 64	3 ± 0	2.98 ± 0.14	2.28 ± 0.09	1.31 ± 0.10	31 ± 10	142 ± 13
	13	431 ± 132	4 ± 0	3.27 ± 0.04	3.11 ± 0.18	1.06 ± 0.05	30 ± 10	156 ± 21
	26	360 ± 160	5 ± 1	3.18 ± 0.07	3.28 ± 0.15	0.97 ± 0.03	31 ± 10	143 ± 17
	52	379 ± 174	5 ± 1	3.22 ± 0.06	3.29 ± 0.14	0.98 ± 0.05	20 ± 3	137 ± 9
15 000	0	478 ± 76	3 ± 0	3.13 ± 0.18	2.30 ± 0.12	1.37 ± 0.09	27 ± 8	142 ± 24
	13	1518 ± 370**	7 ± 1*	3.00 ± 0.18*	3.58 ± 0.35*	0.85 ± 0.12**	45 ± 12*	218 ± 24**
	26	1884 ± 581**	9 ± 3**	2.84 ± 0.18*	3.70 ± 0.51	0.78 ± 0.12**	49 ± 15**	220 ± 29**
	52	2282 ± 1096**	14 ± 7**	2.78 ± 0.19**	3.72 ± 0.41	0.75 ± 0.05**	34 ± 7**	219 ± 30**

Table 22 (continued)

Dietary concentration (ppm)	Week	Group mean values ± standard deviation						
		Alkaline phosphatase (U/l)	GGT (U/l)	Albumin (g/dl)	Globulin (mg/dl)	Albumin to globulin ratio	Triglycerides (mg/dl)	Total cholesterol (mg/dl)
Females								
0	0	423 ± 40	4 ± 1	3.16 ± 0.13	2.30 ± 0.06	1.38 ± 0.09	23 ± 5	142 ± 26
	13	279 ± 98	3 ± 1	3.27 ± 0.08	2.46 ± 0.18	1.33 ± 0.08	16 ± 3	117 ± 12
	26	233 ± 158	6 ± 1	3.28 ± 0.10	2.81 ± 0.13	1.17 ± 0.06	21 ± 8	138 ± 29
	52	244 ± 188	5 ± 1	3.34 ± 0.19	2.90 ± 0.18	1.15 ± 0.09	12 ± 1	130 ± 22
310	0	431 ± 44	3 ± 1	3.02 ± 0.06	2.28 ± 0.24	1.33 ± 0.12	22 ± 6	135 ± 19
	13	294 ± 10	4 ± 1	3.33 ± 0.06	2.68 ± 0.34	1.25 ± 0.14	20 ± 4	139 ± 18
	26	203 ± 33	5 ± 0	3.29 ± 0.10	3.10 ± 0.38	1.07 ± 0.11	26 ± 12	163 ± 47
	52	283 ± 101	6 ± 2	3.36 ± 0.08	3.32 ± 0.25	1.02 ± 0.07	12 ± 2	138 ± 22
2150	0	486 ± 119	4 ± 1	3.13 ± 0.15	2.42 ± 0.09	1.30 ± 0.06	23 ± 9	144 ± 30
	13	441 ± 145	4 ± 1	3.31 ± 0.11	2.94 ± 0.25*	1.14 ± 0.11*	22 ± 6	134 ± 35
	26	351 ± 147	5 ± 1	3.21 ± 0.20	3.28 ± 0.55	1.01 ± 0.23	27 ± 11	145 ± 19
	52	545 ± 310	5 ± 1	3.37 ± 0.05	3.41 ± 0.77	1.03 ± 0.22	13 ± 3	144 ± 33
15 000	0	431 ± 63	3 ± 0	3.12 ± 0.09	2.32 ± 0.13	1.35 ± 0.06	23 ± 13	126 ± 24
	13	2012 ± 998**	9 ± 4*	3.09 ± 0.06*	3.07 ± 0.17*	1.01 ± 0.06**	31 ± 19	163 ± 24
	26	3579 ± 3916**	22 ± 27	2.92 ± 0.16*	3.30 ± 0.21	0.88 ± 0.08	34 ± 17	173 ± 25
	52	4578 ± 5064**	28 ± 31*	2.99 ± 0.21*	3.46 ± 0.31	0.87 ± 0.10	16 ± 6	173 ± 37

From Nakashima (2006). Data extracted from report tables 19-1 to 19-5 (pp. 153–157) and tables 20-1 to 20-5 (pp. 158–162).
GGT, gamma-glutamyltransferase; U, unit; * $P < 0.05$; ** $P < 0.01$

Table 23. Selected individual absolute and relative organ weights in dogs

Dietary concentration (ppm)	Group mean weights ± standard deviation			
	Liver	Adrenal	Heart	Epididymis
Absolute weight (g)				
Males				
0	260 ± 24	1.051 ± 0.103	90.7 ± 13.7	3.301 ± 0.558
310	272 ± 18	1.046 ± 0.033	91.9 ± 9.2	3.346 ± 0.376
2150	282 ± 21	1.084 ± 0.151	80.4 ± 7.0	2.862 ± 0.227
15 000	393 ± 26**	1.370 ± 0.190*	64.4 ± 2.4*	2.379 ± 0.424*
Females				
0	267 ± 35	1.189 ± 0.100	84.2 ± 6.1	—
310	255 ± 6	1.124 ± 0.197	81.5 ± 6.6	—
2150	291 ± 50	1.206 ± 0.345	85.3 ± 12.3	—
15 000	369 ± 59	1.613 ± 0.351	74.6 ± 9.1	—
Relative weight (% of body weight)				
Males				
0	2.46 ± 0.16	0.0101 ± 0.0015	0.87 ± 0.12	0.0316 ± 0.0065
310	2.70 ± 0.14	0.0104 ± 0.0012	0.91 ± 0.07	0.0332 ± 0.0041

Table 23 (continued)

Dietary concentration (ppm)	Group mean weights ± standard deviation			
	Liver	Adrenal	Heart	Epididymis
2150	2.79 ± 0.33	0.0110 ± 0.0031	0.80 ± 0.10	0.0285 ± 0.0051
15 000	4.78 ± 0.24**	0.0166 ± 0.0018**	0.79 ± 0.10	0.0288 ± 0.0034
Females				
0	2.70 ± 0.59	0.0119 ± 0.0014	0.86 ± 0.17	—
310	2.57 ± 0.05	0.0113 ± 0.0020	0.82 ± 0.07	—
2150	2.90 ± 0.36	0.0120 ± 0.0031	0.86 ± 0.12	—
15 000	4.42 ± 0.69	0.0194 ± 0.0042**	0.89 ± 0.05	—

From Nakashima (2006). Data extracted from report tables 21-1 and 21-2 (pp. 167–168) and tables 22-1 and 22-2 (pp. 169–170).
* $P < 0.05$; ** $P < 0.01$

Table 24. Incidences of selected histopathological alterations in dogs

Tissue/lesion	Incidence in group			
	Dietary concentration (ppm)			
	0	310	2150	15 000
Males (*n* = 4)				
Adrenal cortical cell hypertrophy	0	0	0	4*
Liver diffuse hepatocyte hypertrophy	0	0	0	4*
Gall bladder cholecystitis	0	0	0	1
Gall bladder mucosal cell hyperplasia	0	0	0	3
Females (*n* = 4)				
Adrenal cortical cell hypertrophy	0	0	0	4*
Liver diffuse hepatocyte hypertrophy	0	0	0	4*
Gall bladder cholecystitis	0	0	0	1
Gall bladder mucosal cell hyperplasia	0	0	0	3

From Nakashima (2006). Data extracted from report tables 25-1 and 26-1, pp. 173 and 175.
* $P < 0.05$

mucosal epithelial hyperplasia (three males and all females), in some instances accompanied by cholecystitis, in the gall bladder, and slight adrenal cortical cell hypertrophy (all animals) (Table 24). There were no other treatment-related histopathological alterations at any dose level.

The NOAEL was 2150 ppm (equal to 54.4 and 56.6 mg/kg bw per day in males and females, respectively) (Nakashima, 2006), based on the occurrence of reduced weight gain, haematological alterations in males, clinical indicators of hepatic dysfunction and histopathological alterations in the liver, gall bladder and adrenal glands, accompanied by increased organ weights, at 15 000 ppm (equal to 461 and 445 mg/kg bw per day in males and females, respectively).

(b) Dermal application

Rats

In a 28-day dermal toxicity study, groups of 10 Crl:CD(SD) rats of each sex per dose were treated dermally with penthiopyrad (purity 98.6%) applied to approximately 10% of the body surface at a dose level of 0, 40, 200 or 1000 mg/kg bw per day for 4 weeks. The volume of application was 0.2 ml, 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. 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, were 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 investigations performed in week 4 did not indicate any treatment-related effects at any dose level. There were no treatment-related changes in the plasma chemistry profile that were considered to be adverse. There were no treatment-related gross lesions at necropsy at any dose level, and absolute and body weight–adjusted organ weights were unaffected at all dose levels. 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.

The NOAEL was 1000 mg/kg bw per day, the highest dose tested (Groom, 2008a).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a carcinogenicity study in mice, penthiopyrad (purity 99.8%) was administered in the diet to 52 CD-1 mice of each sex per dose at a target dose level of 0, 20, 60, 200 or 600 mg/kg bw per day for 78 weeks (achieved dose levels of 0/0, 19.9/20, 59.8/60.3, 200/201 and 602/604 mg/kg bw per day for males and 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 (10 mice of each sex per group; survivors at week 78 only) 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, Schmorl stain for lipofuscin, Berlin blue for haemosiderin and Fontana-Masson stain for melanin were performed on the thyroids of two animals of each sex from the control and high-dose groups to characterize brown pigment detected in follicular cells of high-dose animals.

There was no statistically significant effect on mortality in either sex at any dose level. Treatment-related clinical signs were confined to females at 600 mg/kg bw per day, in which there were higher incidences of pale-coloured skin and pale-coloured eyelids, compared with control incidences. Reduced body weight and body weight gain occurred in both sexes at 600 mg/kg bw per day, but not at lower dose levels (Table 25). There was no effect on feed consumption in either sex at any dose level. There were no toxicologically relevant alterations at any dose level in the total and differential white blood cell counts in week 78.

Treatment-related gross lesions at necropsy occurred at dose levels of 200 and 600 mg/kg bw per day. Males at 600 mg/kg bw per day sacrificed after 78 weeks showed higher incidences of dark-coloured liver and spots on the liver. Males at 200 mg/kg bw per day showed an increased incidence of liver masses. The livers of male animals at lower dose levels and females in all treated groups were not affected. Treatment-related effects on organ weights occurred at dose levels of 200 and 600 mg/

Table 25. Selected group mean body weight data in mice

Dose (mg/kg bw per day)	Group mean body weight (g) ± standard deviation						Week 78 (% of control)
	Week						
	0	4	13	28	52	78	
Males							
0	32.2 ± 1.6	39.6 ± 2.5	45.0 ± 3.4	48.9 ± 4.6	49.5 ± 5.5	50.0 ± 6.5	—
20	32.1 ± 1.6	39.4 ± 3.0	45.4 ± 5.3	49.5 ± 6.2	49.2 ± 6.4	47.8 ± 7.1	95.6
60	32.1 ± 1.4	39.7 ± 2.3	45.3 ± 3.5	48.9 ± 5.1	49.6 ± 4.7	49.3 ± 5.6	98.6
200	32.1 ± 1.9	39.0 ± 2.0	44.4 ± 2.7	47.5 ± 3.6	47.2 ± 4.8	48.5 ± 4.6	97.0
600	32.1 ± 1.6	39.2 ± 2.2	43.4 ± 3.0	46.0 ± 3.6**	47.1 ± 3.7	46.8 ± 3.5	93.6
Females							
0	26.3 ± 1.6	31.9 ± 3.0	39.1 ± 4.9	47.2 ± 7.0	52.3 ± 8.2	53.5 ± 9.7	—
20	25.9 ± 1.4	31.6 ± 2.7	38.2 ± 4.0	46.2 ± 4.9	51.8 ± 5.5	53.0 ± 5.8	99.1
60	26.1 ± 1.6	32.0 ± 3.4	39.2 ± 5.6	46.3 ± 8.2	51.6 ± 9.2	52.2 ± 9.0	97.6
200	25.8 ± 1.5	31.4 ± 2.7	37.7 ± 5.2	45.5 ± 7.1	49.8 ± 7.6	51.5 ± 8.7	96.3
600	25.9 ± 1.5	31.4 ± 3.0	36.5 ± 4.4*	42.3 ± 6.4**	46.5 ± 6.6**	48.1 ± 6.7**	89.9

From Enomoto (2006)
* $P < 0.05$; ** $P < 0.01$

Table 26. Selected group mean organ weight data in mice

Sex	Organ	Group mean % of control value			
		Dose (mg/kg bw per day)			
		20	60	200	600
Male	Body weight	88*	94	90	87**
	Brain (relative)	113*	107	109	112*
	Thyroid (absolute)	98	108	110	164**
	Thyroid (relative)	113	115	121	188**
	Liver (absolute)	97	118	162**	170**
	Liver (relative)	109	125	178**	191**
	Epididymides (relative)	125**	104	104	117
Female	Body weight	94	93	96	85
	Brain (relative)	103	108	100	116
	Thyroid (absolute)	93	104	130	163**
	Thyroid (relative)	96	105	132	185**
	Liver (absolute)[a]	84	85	92	99
	Liver (relative)[a]	86	88	93	112

From Enomoto (2006)
* $P < 0.05$; ** $P < 0.01$
[a] Excluding very high values shown to be due to spontaneous malignant lymphoma.

Table 27. Treatment-related non-neoplastic alterations in mice

Sex	Organ/lesion	Incidence				
		Dose (mg/kg bw per day)				
		0	20	60	200	600
All animals						
Male	*Thyroid: number examined*	*52*	*52*	*52*	*52*	*52*
	- altered colloid	3	3	6	11*	41**
	- brown pigment (follicular cell)	0	0	0	1	19**
	- hypertrophy (follicular cell)	1	3	4	14**	28**
	Lung: number examined	*52*	*27*	*30*	*29*	*52*
	- alveolar foamy cell accumulation	4	0	3	0	4
Female	*Thyroid: number examined*	*52*	*52*	*52*	*52*	*52*
	- altered colloid	2	5	1	6	44**
	- brown pigment (follicular cell)	0	0	0	0	28**
	- hypertrophy (follicular cell)	0	1	2	12**	34**
	Lung: number examined	*52*	*52*	*52*	*52*	*52*
	- alveolar foamy cell accumulation	4	9	7	9	11*
Animals sacrificed at 78 weeks						
Male	*Thyroid: number examined*	*36*	*32*	*34*	*31*	*34*
	- altered colloid	3	3	6	11**	31**
	- brown pigment (follicular cell)	0	0	0	1	19**
	- hypertrophy (follicular cell)	1	3	4	14**	25**
Female	*Thyroid: number examined*	*42*	*42*	*41*	*40*	*42*
	- altered colloid	2	5	1	6	38**
	- brown pigment (follicular cell)	0	0	0	0	27**
	- hypertrophy (follicular cell)	0	1	2	11**	32**

From Enomoto (2006)
* $P < 0.05$; ** $P < 0.01$

kg bw per day, but not at lower dose levels. Males and females at 600 mg/kg bw per day showed markedly increased absolute and relative thyroid weights, and males at 200 and 600 mg/kg bw per day showed absolute and relative liver weights 62–91% higher than control values (Table 26).

Treatment-related non-neoplastic histopathological alterations occurred in the thyroid of both sexes at 200 and 600 mg/kg bw per day and in the lungs of females at 600 mg/kg bw per day (Table 27). There was a dose-related increase in the incidences of thyroid follicular cell hypertrophy in both sexes at 200 and 600 mg/kg bw per day, accompanied in males at 200 mg/kg bw per day and in both sexes at 600 mg/kg bw per day by altered colloid and in both sexes at 600 mg/kg bw per day by lipofuscin deposition. Females at 600 mg/kg bw per day also showed an increased incidence of alveolar foamy cell accumulation, often associated with lymphocyte infiltration. All other statistically significant differences in the incidences of non-neoplastic alterations were either decreased incidences or showed no dose–response relationship.

Males at 200 and 600 mg/kg bw per day sacrificed after 78 weeks of treatment had significantly higher incidences of hepatocellular adenomas and adenoma plus carcinoma than the contemporary control group, although the incidences of carcinoma alone were not significantly increased in either group (Table 28). The incidences of hepatocellular adenomas and adenoma plus carcinoma were also significantly higher in all males (incidental deaths and terminal sacrifice) at 600 mg/kg bw per day. Although the total incidences of hepatocellular adenoma in males at 200 and 600 mg/kg bw per

Table 28. Treatment-related neoplastic alterations in mice

Sex	Organ/lesion	Incidence				
		Dose (mg/kg bw per day)				
		0	20	60	200	600
All animals						
Male	*Liver: number examined*	*52*	*52*	*52*	*52*	*52*
	- hepatocellular adenoma	7	13	10	13	15*
	- hepatocellular carcinoma	2	1	1	5	6
	- total (adenoma + carcinoma)	9	14	11	15	19*
Female	*Liver: number examined*	*52*	*52*	*52*	*52*	*52*
	- hepatocellular adenoma	4	2	2	4	2
	- hepatocellular carcinoma	0	0	0	0	0
	- total (adenoma + carcinoma)	4	2	2	4	2
Animals sacrificed at 78 weeks						
Male	*Liver: number examined*	*36*	*32*	*34*	*31*	*34*
	- hepatocellular adenoma	5	8	7	11*	12*
	- hepatocellular carcinoma	1	1	1	4	2
	- total (adenoma + carcinoma)	6	9	8	13*	13*
Female	*Liver: number examined*	*42*	*42*	*41*	*40*	*42*
	- hepatocellular adenoma	4	2	2	4	2
	- hepatocellular carcinoma	0	0	0	0	0
	- total (adenoma + carcinoma)	4	2	2	4	2

From Enomoto (2006)
* $P < 0.05$

Table 29. Historical control data for hepatocellular adenoma and carcinoma in ICR (Crj:CD-1) strain mice

Lesion	Control incidence												
	Year study started												
	1995	1996	1999	2001	2002	2003	2003	2004	2004	2005	2005	2006	2006
Males													
Number examined	*50*	*52*	*52*	*52*	*52*	*52*	*52*	*51*	*56*	*52*	*52*	*56*	*52*
Hepatocellular adenoma	14	12	17	9	14	16	14	14	16	18	15	18	14
Hepatocellular carcinoma	0	2	3	3	4	1	0	3	1	2	5	3	1
Hepatocellular adenoma and/or carcinoma	14	13	19	12	15	16	14	16	16	19	19	19	15

From Enomoto (2006)

day were higher than the concurrent control incidence, they were within the historical control incidence range, whereas the contemporary control incidence was lower than the historical control range (Table 29). Furthermore, there was no clear dose–response relationship, no increase in the incidence of preneoplastic alterations, no similar effect in females and no effect on tumour latency.

There were no other statistically significant differences between treated and control male groups in the incidences of individual tumour types. Statistically significant differences between treated and control female groups in the incidences of individual tumour types were confined to lower incidences in the treated groups.

The NOAEL was 60 mg/kg bw per day, based on the occurrence of non-neoplastic histopathological alterations in the thyroid of both sexes at 200 mg/kg bw per day (Enomoto, 2006). Similar effects were observed at 600 mg/kg bw per day, as well as increased thyroid weights in both sexes, decreased body weight gain in both sexes, clinical signs (pale skin and eyelids) in females, gross liver pathology in males and lung histopathology in females.

Rats

In a chronic oral toxicity study, penthiopyrad (purity 98.8%) was administered in the diet to 20 Wistar rats of each sex per dose at a target dose level of 0, 6.25, 25, 100 or 400 mg/kg bw per day for 52 weeks. Additional groups of 10 male and 10 female animals, similarly treated, were sacrificed after 26 weeks of treatment. Clinical signs were recorded daily, and a detailed physical examination was performed weekly. Body weights and feed consumption were recorded weekly for the first 13 weeks and every 2 weeks thereafter. Ophthalmoscopic examinations were performed pre-dosing, on all animals scheduled to be sacrificed after 52 weeks and on 10 animals of each sex in animals treated at 0 or 400 mg/kg bw per day in week 50. Haematology, clinical chemistry and urinalysis were performed on 10 animals of each sex per group in week 26 and on all surviving animals in week 50. 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 sacrificed after 52 weeks, premature decedents from all groups, gross lesions from all animals, and liver and thyroid gland from all animals sacrificed after 26 weeks were processed and examined by light microscopy. Adrenals, kidneys, liver, thyroid and ovaries from animals in the low- and intermediate-dose groups sacrificed after 52 weeks and from all animals sacrificed after 26 weeks were also processed and examined by light microscopy. Data were analysed statistically where appropriate.

There were no treatment-related deaths, clinical signs or effects on feed consumption at any dose level. Reduced body weight gain occurred only in males treated at 400 mg/kg bw per day (Table 30). Treatment-related effects on the haematological profile at 26 weeks were confined to increased partial thromboplastin time and relative prothrombin time in both sexes at 400 mg/kg bw per day and increased haemoglobin distribution width in females at 100 and 400 mg/kg bw per day.

Table 30. Selected group mean body weights in rats sacrificed after 52 weeks

Dose (mg/kg bw per day)	Group mean body weight (g)							Body weight gain (% of control)
	Week							
	0	2	4	13	28	40	52	52
Males								
0	164 ± 7.2	255 ± 12.4	307 ± 15.5	422 ± 26.7	494 ± 34.2	528 ± 39.5	565 ± 49.9	—
6.25	161 ± 6.5	256 ± 15.3	316 ± 21.3	443 ± 39.1	527 ± 49.0*	560 ± 53.7	592 ± 60.1	109.8
25	162 ± 7.1	256 ± 12.0	311 ± 20.3	427 ± 34.8	514 ± 44.9	548 ± 51.5	578 ± 71.1	105.2
100	161 ± 7.0	247 ± 12.0	293 ± 18.3*	399 ± 33.5	484 ± 45.8	518 ± 52.9	555 ± 57.7	99.7
400	166 ± 7.0	246 ± 11.9	296 ± 14.3	403 ± 28.0	473 ± 32.2	508 ± 38.6	533 ± 37.2	90.0
Females								
0	134 ± 8.5	173 ± 11.1	199 ± 13.9	248 ± 19.0	270 ± 24.6	286 ± 27.1	304 ± 42.6	—
6.25	135 ± 9.2	176 ± 10.0	206 ± 15.2	255 ± 16.4	280 ± 20.2	294 ± 23.5	307 ± 29.5	100.7
25	134 ± 6.5	177 ± 8.2	203 ± 9.0	255 ± 10.5	279 ± 15.6	290 ± 19.8	308 ± 29.1	103.4
100	134 ± 8.2	174 ± 12.2	202 ± 12.8	254 ± 15.3	278 ± 17.2	285 ± 20.3	300 ± 24.5	98.4
400	132 ± 6.7	173 ± 18.1	197 ± 11.7	247 ± 16.5	270 ± 17.2	282 ± 21.5	293 ± 27.9	97.3

From Blumbach (2006a)
* $P < 0.05$

Table 31. Selected group mean haematology data in rats sacrificed after 26 or 52 weeks

Dose (mg/kg bw per day)	Group mean value					
	MCV (fl)	MCH (fmol)	MCHC (mmol/l)	MetHb (rel.)	PT (rel.)	PTT (s)
Males						
26 weeks						
0	51.54 ± 1.02	1.074 ± 0.020	20.828 ± 0.171	0.0080 ± 0.0014	0.967 ± 0.32	18.94 ± 2.39
6.25	52.18 ± 2.22	1.104 ± 0.056	21.178 ± 0.255*	0.0078 ± 0.0011	1.004 ± 0.73	16.92 ± 3.25
25	51.84 ± 0.97	1.092 ± 0.032	21.068 ± 0.272	0.0077 ± 0.0012	1.034 ± 0.70*	17.31 ± 2.76
100	52.20 ± 1.04	1.092 ± 0.031	20.927 ± 0.258	0.0081 ± 0.0013	1.009 ± 0.32	17.92 ± 2.42
400	50.06 ± 2.02	1.020 ± 0.053	20.380 ± 0.305**	0.0078 ± 0.0009	1.032 ± 0.52*	24.63 ± 3.48**
52 weeks						
0	50.86 ± 1.90	1.082 ± 0.050	21.253 ± 0.267	0.0091 ± 0.0024	0.856 ± 0.066	14.76 ± 3.12
6.25	52.93 ± 3.37*	1.127 ± 0.045*	21.314 ± 0.264	0.0075 ± 0.0028	0.847 ± 0.061	14.44 ± 2.33
25	52.36 ± 2.09	1.131 ± 0.053**	21.619 ± 0.253**	0.0074 ± 0.0020	0.876 ± 0.071	15.88 ± 3.97
100	51.54 ± 2.34	1.100 ± 0.057	21.331 ± 0.268	0.0074 ± 0.0014	0.874 ± 0.074	16.11 ± 4.04
400	48.99 ± 1.39*	1.021 ± 0.036**	20.840 ± 0.298**	0.0085 ± 0.0022	0.780 ± 0.056**	21.41 ± 5.16**
Females						
26 weeks						
0	57.03 ± 1.12	1.218 ± 0.036	21.370 ± 0.473	0.0051 ± 0.0026	1.039 ± 0.062	16.17 ± 2.57
6.25	56.05 ± 3.04	1.197 ± 0.065	21.329 ± 0.273	0.0059 ± 0.0014	1.073 ± 0.046	17.34 ± 2.09
25	55.29 ± 1.73	1.181 ± 0.045	21.358 ± 0.240	0.0070 ± 0.0015	1.070 ± 0.060	17.40 ± 2.85
100	55.17 ± 1.46	1.167 ± 0.033	21.169 ± 0.206	0.0081 ± 0.0015**	1.098 ± 0.082	19.08 ± 2.64
400	54.29 ± 1.18**	1.143 ± 0.022**	21.038 ± 0.235	0.0077 ± 0.0020*	1.229 ± 0.067**	20.15 ± 4.04*
52 weeks						
0	52.72 ± 1.64	1.217 ± 0.045	23.084 ± 0.382	0.0086 ± 0.0021	0.781 ± 0.045	14.74 ± 2.71
6.25	52.49 ± 1.07	1.207 ± 0.033	22.958 ± 0.318	0.0073 ± 0.0019	0.821 ± 0.055*	14.01 ± 2.97
25	52.69 ± 1.85	1.227 ± 0.057	23.286 ± 0.421	0.0066 ± 0.0016*	0.839 ± 0.055**	14.84 ± 2.97
100	53.43 ± 2.68	1.233 ± 0.063	23.076 ± 0.295	0.0071 ± 0.0015	0.838 ± 0.050**	14.23 ± 2.16
400	50.96 ± 1.33**	1.175 ± 0.035*	23.063 ± 0.396	0.0086 ± 0.0017	0.919 ± 0.043**	16.35 ± 4.02

From Blumbach (2006a)

MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; MetHb, methaemoglobin; PT, prothrombin time; PTT, partial thromboplastin time; rel., relative; * $P < 0.05$; ** $P < 0.01$

At 52 weeks, increased partial thromboplastin time, slightly reduced relative prothrombin time and slightly reduced relative reticulocyte count occurred in males at 400 mg/kg bw per day (Table 31).

Treatment-related effects on the clinical chemistry profile after 26 or 50 weeks occurred in one or both sexes at 100 and/or 400 mg/kg bw per day. Increased levels of cholesterol, phospholipids, potassium ion, globulin, GGT and alkaline phosphatase, reduced levels of glutamate dehydrogenase and glucose, and reduced albumin to globulin ratio occurred at 400 mg/kg bw per day. Effects at 100 mg/kg bw per day were confined to increased plasma globulin and decreased albumin to globulin ratio (Table 32). There were no treatment-related alterations in the urinalysis profile at any dose level.

Treatment-related changes in organ weight data occurred at dose levels of 100 and 400 mg/kg bw per day. Liver weights of both sexes at 100 and 400 mg/kg bw per day were increased after 26 and 52 weeks of treatment. Males at 100 and 400 mg/kg bw per day showed higher adrenal weights than the controls after 26 weeks of treatment, but not after 52 weeks. Conversely, females at 400 mg/kg bw per day showed slightly higher adrenal weights after 52 weeks, but not after 26 weeks. The kidney weights of males at 100 and 400 mg/kg bw per day were increased after 26 weeks of treatment, but only the relative kidney weight at 400 mg/kg bw per day remained increased after 52 weeks (Table 33).

Table 32. Selected group mean plasma chemistry data in rats sacrificed after 26 or 50 weeks

Dose (mg/kg bw per day)	Group mean value						
	Bilirubin (µmol/l)	Cholesterol (mmol/l)	Phospholipid (mmol/l)	GLDH (U/l)	AP (U/l)	GGT (U/l)	A/G
Males							
Week 27							
0	1.738 ± 0.343	2.045 ± 0.462	1.593 ± 0.280	8.41 ± 3.69	46.54 ± 8.83	0.00	1.526 ± 0.179
6.25	1.438 ± 0.190*	2.136 ± 0.377	1.687 ± 0.195	6.27 ± 1.63	47.60 ± 13.48	0.00	1.531 ± 0.100
25	1.489 ± 0.309	2.185 ± 0.494	1.637 ± 0.254	7.17 ± 2.33	43.65 ± 12.09	0.00	1.484 ± 0.147
100	1.349 ± 0.164**	2.311 ± 0.275	1.814 ± 0.185	5.59 ± 1.70	47.95 ± 6.12	0.00	1.479 ± 0.140
400	1.252 ± 0.225**	2.707 ± 0.245**	2.079 ± 0.195**	3.66 ± 1.23**	62.19 ± 11.40**	8.25 ± 3.46**	1.514 ± 0.134
Week 51							
0	1.946 ± 0.428	2.648 ± 0.428	1.878 ± 0.254	12.17 ± 9.00	53.35 ± 10.19	0.00	1.535 ± 0.154
6.25	1.689 ± 0.325	2.520 ± 0.523	1.862 ± 0.321	10.40 ± 7.11	51.17 ± 13.15	0.00	1.558 ± 0.178
25	1.635 ± 0.322*	2.592 ± 0.498	1.895 ± 0.294	10.19 ± 6.34	50.29 ± 9.24	0.00	1.639 ± 0.179
100	1.566 ± 0.296**	2.718 ± 0.547	2.007 ± 0.324	12.72 ± 11.36	50.51 ± 11.23	0.92 ± 4.11	1.635 ± 0.183
400	1.457 ± 0.252**	3.198 ± 0.511**	2.279 ± 0.322**	4.44 ± 1.63**	63.73 ± 13.85*	10.56 ± 3.63**	1.581 ± 0.006
Females							
Week 27							
0	2.702 ± 0.652	2.006 ± 0.430	2.022 ± 0.312	23.95 ± 20.26	15.75 ± 3.30	0.00	2.178 ± 0.191
6.25	2.347 ± 0.293	2.212 ± 0.507	2.158 ± 0.334	22.52 ± 33.67	15.16 ± 2.60	0.00	2.021 ± 0.238
25	2.163 ± 0.516	1.883 ± 0.407	1.939 ± 0.335	21.28 ± 23.09	17.09 ± 3.69	0.00	1.948 ± 0.186*
100	1.591 ± 0.306**	2.077 ± 0.442	2.024 ± 0.384	13.51 ± 15.76	14.44 ± 2.72	0.00	1.906 ± 0.135**
400	1.420 ± 0.232**	3.206 ± 0.578**	2.660 ± 0.411**	6.65 ± 9.05**	13.54 ± 3.20	3.78 ± 1.55**	1.699 ± 0.114**
Week 51							
0	2.888 ± 0.713	2.075 ± 0.665	2.017 ± 0.459	48.63 ± 83.64	15.61 ± 4.07	0.00	2.082 ± 0.191
6.25	2.399 ± 0.648	2.112 ± 0.476	2.044 ± 0.370	31.52 ± 36.01	15.93 ± 4.92	0.00	2.131 ± 0.171
25	2.634 ± 0.964	2.463 ± 0.569	2.292 ± 0.452	83.88 ± 164.68	15.20 ± 4.31	0.35 ± 1.59	2.012 ± 0.217
100	2.024 ± 0.296**	2.809 ± 0.770*	2.579 ± 0.558**	58.17 ± 130.41	13.35 ± 2.53	0.00	2.004 ± 0.182
400	1.852 ± 0.577**	3.761 ± 1.216**	3.130 ± 0.001**	23.92 ± 34.08	13.51 ± 2.88	4.20 ± 3.17**	1.867 ± 0.200**

From Blumbach (2006a)

A/G, albumin to globulin ratio; AP, alkaline phosphatase; GGT, gamma-glutamyltransferase; GLDH, glutamate dehydrogenase; U, unit; * $P < 0.05$; ** $P < 0.01$

Table 33. Selected group mean organ weights in rats sacrificed after 26 or 52 weeks

Dose (mg/kg bw per day)	Group mean values					
	Liver weight		Adrenal weight		Kidney weight	
	g	% of body weight	g	% of body weight	g	% of body weight
Males						
Week 26						
0	10.18 ± 0.79	2.19 ± 0.13	0.054 ± 0.008	0.012 ± 0.001	2.15 ± 0.15	0.46 ± 0.03
6.25	10.25 ± 0.76	2.24 ± 0.20	0.057 ± 0.005	0.013 ± 0.001	2.26 ± 0.18	0.49 ± 0.06
25	10.84 ± 1.58	2.38 ± 0.24	0.054 ± 0.008	0.012 ± 0.001	2.31 ± 0.30	0.51 ± 0.05
100	11.98 ± 1.95*	2.61 ± 0.35**	0.063 ± 0.012	0.014 ± 0.002**	2.42 ± 0.31*	0.53 ± 0.05**
400	12.65 ± 1.12**	2.94 ± 0.11**	0.063 ± 0.009	0.015 ± 0.001**	2.27 ± 0.21	0.53 ± 0.03**
Week 52						
0	14.14 ± 1.75	2.53 ± 0.21	0.051 ± 0.006	0.009 ± 0.001	2.40 ± 0.22	0.43 ± 0.04
6.25	14.72 ± 1.53	2.53 ± 0.22	0.055 ± 0.007	0.009 ± 0.001	2.47 ± 0.29	0.43 ± 0.06
25	13.89 ± 1.81	2.43 ± 0.19	0.052 ± 0.007	0.009 ± 0.002	2.41 ± 0.27	0.42 ± 0.04
100	15.06 ± 2.12	2.75 ± 0.27**	0.051 ± 0.006	0.009 ± 0.001	2.40 ± 0.23	0.44 ± 0.05
400	17.13 ± 1.49**	3.24 ± 0.20**	0.052 ± 0.006	0.010 ± 0.001	2.56 ± 0.24	0.48 ± 0.04**
Females						
Week 26						
0	7.44 ± 0.71	2.89 ± 0.17	0.066 ± 0.008	0.026 ± 0.003	1.46 ± 0.18	0.57 ± 0.06
6.25	8.13 ± 0.80	3.09 ± 0.26	0.071 ± 0.008	0.027 ± 0.002	1.60 ± 0.16	0.61 ± 0.05
25	7.69 ± 0.45	2.97 ± 0.23	0.067 ± 0.005	0.026 ± 0.002	1.55 ± 0.08	0.60 ± 0.05
100	8.34 ± 1.02	3.21 ± 0.18*	0.075 ± 0.012	0.029 ± 0.005	1.52 ± 0.13	0.59 ± 0.04
400	9.56 ± 9.56**	3.87 ± 0.35**	0.068 ± 0.010	0.028 ± 0.004	1.49 ± 0.14	0.61 ± 0.06
Week 52						
0	7.93 ± 1.23	2.66 ± 0.33	0.062 ± 0.011	0.021 ± 0.003	1.63 ± 0.19	0.55 ± 0.05
6.25	8.13 ± 0.70	2.72 ± 0.25	0.064 ± 0.012	0.022 ± 0.005	1.65 ± 0.15	0.55 ± 0.06
25	8.36 ± 0.94	2.81 ± 0.29	0.062 ± 0.007	0.021 ± 0.003	1.65 ± 0.16	0.55 ± 0.05
100	8.89 ± 1.07*	3.05 ± 0.31**	0.066 ± 0.011	0.023 ± 0.005	1.60 ± 0.18	0.55 ± 0.07
400	11.37 ± 1.16**	3.97 ± 0.27**	0.069 ± 0.010	0.024 ± 0.005*	1.64 ± 0.15	0.58 ± 0.05

From Blumbach (2006a)
* $P < 0.05$; ** $P < 0.01$

There were no treatment-related macroscopic findings at necropsy at any dose level after 26 or 52 weeks of treatment. Treatment-related histopathological alterations after 26 weeks of treatment occurred in the liver, thyroid and adrenal glands of both sexes at 400 mg/kg bw per day and in the adrenal glands of females at 100 mg/kg bw per day, but not at lower dose levels. Effects comprised increased incidence and severity of adrenal diffuse hypertrophy in the zona glomerulosa and cortical lipid vacuolation, an increased incidence of diffuse hypertrophy of the thyroid follicular epithelium and an increased amount of periportal fat vacuolation. Periportal cell swelling (hydropic degeneration) occurred in some males (Table 34). Treatment-related histopathological alterations after 52 weeks of treatment occurred in the liver, thyroid and adrenal glands of both sexes, as well as in the ovaries, at 400 mg/kg bw per day and in the adrenal and thyroid glands at 100 mg/kg bw per day, but not at lower dose levels. The effects on the adrenals, thyroid and liver were similar to those at 26 weeks, and in females, the incidence and severity of ovarian interstitial cell hypertrophy were increased (Table 35).

Table 34. Treatment-related histopathological findings in rats sacrificed after 26 weeks

Organ/lesion/severity	Incidence				
	Dose (mg/kg bw per day)				
	0	6.25	25	100	400
Males					
Adrenal gland: number examined	*10*	*10*	*9*	*10*	*10*
Cortical lipid vacuolation					
- minimal	6	6	4	1	0
- slight	1	4	3	5	4
- moderate	2	0	2	3	5
- marked	0	0	0	0	1
- total	9	10	9	9	10^{++}**
Diffuse hypertrophy of zona glomerulosa					
- minimal	1	4	2	2	4
- slight	0	0	0	2	2
- total	1	4	2	4	6^{+}
Thyroid gland: number examined	*10*	*10*	*9*	*10*	*10*
Diffuse follicular hypertrophy					
- minimal	1	3	2	3	1
- slight	2	0	1	4	5
- moderate	0	0	0	0	1
- total	3	3	3	7	7
Liver: number examined	*10*	*10*	*9*	*10*	*10*
Periportal fat vacuolation					
- minimal	5	10	8	5	0
- slight	2	0	0	2	2
- moderate	0	0	0	0	6
- marked	0	0	0	0	2
- total	7	10	8	7	10^{+++}***
Periportal cell swelling					
- minimal	0	0	0	0	4
- slight	0	0	0	0	2
- moderate	0	0	0	0	1
- total	0	0	0	0	7^{++}***
Females					
Adrenal gland: number examined	*10*	*10*	*10*	*10*	*10*
Cortical lipid vacuolation					
- minimal	3	3	1	2	4
- slight	1	0	0	0	6
- total	4	3	1	2	10^{++}***
Diffuse hypertrophy of zona glomerulosa					
- minimal	0	0	1	2	4

Table 34 (continued)

Organ/lesion/severity	Incidence				
	Dose (mg/kg bw per day)				
	0	6.25	25	100	400
- slight	0	0	0	2	3
- total	0	0	1	4^{+}	7^{++}***
Thyroid gland: number examined	*10*	*10*	*10*	*10*	*10*
Diffuse follicular hypertrophy					
- minimal	0	0	2	2	6
- slight	0	0	0	1	3
- total	0	0	2	3	9^{+++}***

From Blumbach (2006a)
Incidence: $^{+}$ $P < 0.05$; $^{++}$ $P < 0.01$; $^{+++}$ $P < 0.001$
Trend: ** $P < 0.01$; *** $P < 0.001$

Table 35. Treatment-related histopathological findings in rats sacrificed after 52 weeks and premature decedents

Organ/lesion/severity	Incidence				
	Dose (mg/kg bw per day)				
	0	6.25	25	100	400
Males					
Adrenal gland: number examined	*20*	*20*	*21*	*20*	*20*
Cortical lipid vacuolation					
- minimal	6	3	8	2	1
- slight	10	11	5	4	4
- moderate	4	6	6	13	14
- marked	0	0	0	1	1
- total	20	20	19	20^{++}	20^{+++}***
Diffuse hypertrophy of zona glomerulosa					
- minimal	3	0	4	11	8
- slight	1	0	0	1	9
- total	4	0	4	12^{+}	17^{+++}***
Thyroid gland: number examined	*20*	*20*	*21*	*20*	*20*
Diffuse follicular hypertrophy					
- minimal	5	10	6	6	5
- slight	4	2	0	7	13
- moderate	0	0	0	0	2
- total	9	12	6	13	20^{+++}***
Liver: number examined	*20*	*20*	*19*	*20*	*20*
Periportal fat vacuolation					
- minimal	0	0	0	2	2
- slight	0	0	0	0	6
- moderate	0	0	0	0	11
- marked	0	0	0	0	1

Table 35 (continued)

Organ/lesion/severity	Incidence				
	Dose (mg/kg bw per day)				
	0	6.25	25	100	400
- total	0	0	0	2	20^{+++}***
Periportal cell swelling					
- minimal	0	0	0	0	5
- slight	0	0	0	0	9
- moderate	0	0	0	0	2
- total	0	0	0	0	16^{+++}***
Periportal single-cell necrosis					
- minimal	0	0	0	0	2
- slight	0	0	0	0	2
- total	0	0	0	0	4^{+}**
Females					
Adrenal gland: number examined	*20*	*20*	*20*	*20*	*20*
Cortical lipid vacuolation					
- minimal	4	4	4	6	2
- slight	0	1	3	5	7
- moderate	0	0	0	1	11
- total	4	5	7	12^{++}	20^{+++}***
Diffuse hypertrophy of zona glomerulosa					
- minimal	1	0	1	3	5
- slight	0	0	0	2	8
- moderate	0	0	0	0	3
- total	1	0	1	5	16^{+++}***
Thyroid gland: number examined	*20*	*20*	*20*	*20*	*20*
Diffuse follicular hypertrophy					
- minimal	1	1	3	6	8
- slight	0	0	0	0	12
- total	1	1	3	6^{+}	20^{+++}***
Ovaries: number examined	*20*	*20*	*20*	*20*	*20*
Interstitial cell hypertrophy					
- minimal	4	2	3	4	3
- slight	1	0	2	3	12
- moderate	0	0	0	0	2
- total	5	2	5	7	17^{+++}***
Liver: number examined	*20*	*20*	*20*	*20*	*20*
Centrilobular hypertrophy					
- minimal	0	0	0	0	8
- total	0	0	0	0	8^{++}***

From Blumbach (2006a)
Incidence: $^{+}$ $P < 0.05$; $^{++}$ $P < 0.01$; $^{+++}$ $P < 0.001$
Trend: ** $P < 0.01$; *** $P < 0.001$

Table 36. Selected group mean body weight data in rats

Dose (mg/kg bw per day)	Group mean body weight (g)						% of control
	Week						
	1	13	26	52	78	104	104
Males							
0	162 ± 11.9	404 ± 50.1	483 ± 68.6	571 ± 91.8	636 ± 113.9	681 ± 135.9	—
9	164 ± 13.7	422 ± 40.1	495 ± 49.0	584 ± 66.2	658 ± 83.5	716 ± 108.9	105.1
27	159 ± 11.6	415 ± 39.9	493 ± 46.0	587 ± 58.8	656 ± 75.7	698 ± 94.3	102.5
83	162 ± 13.2	430 ± 46.4**	508 ± 59.0	604 ± 81.7	666 ± 81.5	718 ± 91.4	105.4
250	166 ± 10.7	417 ± 31.0	494 ± 41.7	583 ± 60.3	643 ± 71.4	655 ± 74.9	96.2
Females							
0	124 ± 8.4	248 ± 16.9	277 ± 21.9	313 ± 34.7	349 ± 52.2	391 ± 70.7	—
9	121 ± 9.9	243 ± 16.3	268 ± 20.6	302 ± 40.0	345 ± 55.1	371 ± 56.0	94.9
27	122 ± 9.1	247 ± 17.3	274 ± 19.9	307 ± 35.2	352 ± 49.3	380 ± 63.6	97.2
83	121 ± 10.0	243 ± 16.6	264 ± 16.7**	291 ± 21.7**	325 ± 33.5	363 ± 45.6	92.8
250	122 ± 9.3	248 ± 17.6	273 ± 21.1	303 ± 31.4	335 ± 49.9	358 ± 49.1*	91.6

From Blumbach (2006b)
* $P < 0.05$; ** $P < 0.01$

The NOAEL was 25 mg/kg bw per day, based on the occurrence of an altered plasma chemistry profile, increased liver weight and histopathological alterations in the adrenal and thyroid glands at 100 mg/kg bw per day. Haematological alterations, reduced body weight gain and histopathological alterations in liver and ovaries were observed at 400 mg/kg bw per day (Blumbach, 2006a).

In a carcinogenicity study in rats, penthiopyrad (purity 99.8%) was administered in the diet to 50 Wistar rats of each sex per dose at a target dose level of 0, 9, 27, 83 or 250 mg/kg bw per day for 104 weeks. 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 2 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. Data were analysed statistically where appropriate.

There was no treatment-related effect at any dose level on the survival of either sex. There were no treatment-related clinical signs in either sex at any dose level. Male body weight was unaffected by treatment at all dose levels, but reduced body weight gain occurred in females treated at 83 and 250 mg/kg bw per day (Table 36). There was no treatment-related effect at any dose level on feed consumption. There were no treatment-related effects at any dose level on total red and white blood cell counts or differential white blood cell counts. Treatment-related effects on organ weights and ratios were confined to increased liver weight in both sexes at 250 mg/kg bw per day (Table 37). Macroscopic lesions identified at necropsy that were considered treatment related occurred in the liver (accentuated lobular pattern), thyroid (nodules), lungs (foci) and adrenal gland (discoloration) in one or both sexes at 250 mg/kg bw per day and in the lungs of females at 83 mg/kg bw per day (Table 38). Adverse

Table 37. Group mean liver weights in rats sacrificed after 104 weeks in rats

Dose (mg/kg bw per day)	Group mean liver weights		
	g	% of body weight	% of brain weight
Males			
0	17.27 ± 4.08	2.52 ± 0.39	782.06 ± 171.32
9	17.58 ± 3.16	2.55 ± 0.35	786.40 ± 129.80
27	17.56 ± 3.30	2.63 ± 0.42	784.03 ± 140.88
83	18.12 ± 2.65	2.66 ± 0.33	802.35 ± 112.60
250	18.89 ± 3.41	2.98 ± 0.35**	860.72 ± 155.28
Females			
0	9.98 ± 2.00	2.68 ± 0.39	496.84 ± 97.65
9	9.81 ± 1.88	2.79 ± 0.35	482.73 ± 99.17
27	10.21 ± 2.79	2.80 ± 0.63	495.05 ± 122.74
83	9.79 ± 1.57	2.85 ± 0.39	490.70 ± 77.14
250	11.37 ± 2.53*	3.36 ± 0.50**	565.90 ± 124.72*

From Blumbach (2006b)
* $P < 0.05$; ** $P < 0.01$

Table 38. Incidences of selected gross lesions identified at necropsy: all rats

Organ/lesion	Incidence				
	Dose (mg/kg bw per day)				
	0	9	27	83	250
Males					
No. of animals examined	*50*	*50*	*50*	*50*	*50*
Liver: accentuated lobular pattern	1	1	0	1	8*
Thyroid: nodules	2	0	1	0	5
Adrenal gland: discoloration	0	0	0	0	1
Lungs: focus/foci	5	5	3	4	6
Seminal vesicles: reduced in size	1	3	4	1	7*
Females					
No. of animals examined	*50*	*50*	*50*	*50*	*50*
Liver: accentuated lobular pattern	1	0	0	0	0
Thyroid: nodules	0	1	2	0	0
Adrenal gland: discoloration	0	0	0	2	5*
Lungs: focus/foci	5	4	4	13*	13*
Uterus: thickened	2	3	9*	9*	9*

From Blumbach (2006b)
* $P < 0.05$

treatment-related non-neoplastic lesions occurred in the liver (hypertrophy, periportal fatty degeneration, fatty change), lungs (interstitial inflammation) and adrenal glands (focal fatty change). Effects on the kidneys (interstitial fibrosis and renal glomerulosclerosis) were observed at doses of 27 mg/kg bw per day and above in males; however, they were considered to be related to the presence of chronic progressive nephropathy and not to be treatment related. There were no other non-neoplastic findings considered to be treatment related, and none occurred in animals treated at 9 mg/kg bw per day (Table 39).

Table 39. Incidences of selected non-neoplastic histopathological alterations: all rats

Organ/lesion	Incidence (mean severity)				
	Dose (mg/kg bw per day)				
	0	9	27	83	250
Males					
Liver					
No. examined	*50*	*50*	*50*	*50*	*50*
- hypertrophy	1 (2.0)	2 (2.0)	1 (1.0)	6 (1.7)	11** (1.5)
- fatty change	37 (1.4)	39 (1.2)	45* (1.2)	36 (1.2)	28 (1.4)
- periportal fatty degeneration	0 (—)	0 (—)	0 (—)	7** (1.7)	33*** (2.1)
Kidneys					
No. examined	*50*	*50*	*50*	*50*	*50*
- lipofuscin	1 (1.0)	0 (—)	0 (—)	1 (1.0)	4 (1.0)
- tubular basophilia	37 (1.9)	44 (1.8)	38 (1.9)	38 (1.9)	45* (2.1)
- interstitial fibrosis	6 (1.5)	13 (1.3)	17** (1.1)	20** (1.5)	17** (1.6)
- pyelitis	2 (2.0)	8* (1.4)	4 (1.3)	10* (1.7)	9* (1.4)
- glomerulosclerosis	8 (1.3)	12 (1.3)	21** (1.2)	23** (1.5)	23** (1.3)
- chronic nephropathy	28 (2.1)	41** (1.8)	34 (2.0)	35 (2.0)	41** (2.1)
Lungs					
No. examined	*50*	*50*	*50*	*49*	*50*
- alveolar macrophages	17 (1.1)	22 (1.1)	17 (1.2)	26* (1.4)	13 (1.2)
- interstitial inflammation	2 (1.0)	1 (2.0)	2 (1.5)	1 (1.0)	5 (1.2)
Adrenals					
No. examined	*49*	*50*	*50*	*50*	*50*
- cortical atrophy	1 (2.0)	2 (2.0)	0 (—)	1 (2.0)	4 (1.8)
- focal fatty change	16 (1.3)	20 (1.3)	21 (1.4)	24 (1.3)	21 (1.1)
- diffuse hypertrophy	1 (2.0)	2 (2.0)	9** (1.6)	3 (2.3)	1 (2.0)
Females					
Liver					
No. examined	*50*	*50*	*49*	*50*	*47*
- hypertrophy	0 (—)	2 (1.5)	5* (1.8)	5* (2.0)	7** (2.0)
- fatty change	8 (1.8)	8 (2.1)	10 (1.7)	10 (1.0)	26*** (1.3)
- periportal fatty degeneration	0 (—)	0 (—)	0 (—)	0 (—)	0 (—)
Kidneys					
No. examined	*50*	*50*	*49*	*50*	*49*
- lipofuscin	15 (1.1)	12 (1.0)	15 (1.1)	22 (1.1)	20 (1.2)
- tubular basophilia	38 (1.2)	32 (1.2)	34 (1.3)	38 (1.1)	32 (1.2)
- interstitial fibrosis	3 (2.0)	2 (1.0)	4 (1.5)	2 (1.0)	3 (1.3)
- pyelitis	4 (1.5)	6 (1.7)	5 (1.0)	6 (1.0)	4 (1.3)
- glomerulosclerosis	2 (1.0)	4 (1.0)	5 (1.0)	4 (1.0)	6 (1.2)
- chronic nephropathy	36 (1.2)	27 (1.4)	29 (1.4)	30 (1.1)	24 (1.3)
Lungs					
No. examined	*50*	*50*	*49*	*50*	*48*
- alveolar macrophages	6 (1.3)	21*** (1.2)	19** (1.2)	21*** (1.1)	16* (1.6)
- interstitial inflammation	0 (—)	0 (—)	3 (1.7)	3 (1.0)	6* (1.5)

Table 39 (continued)

Organ/lesion	Incidence (mean severity)				
	Dose (mg/kg bw per day)				
	0	9	27	83	250
Ovaries					
No. examined	*50*	*50*	*49*	*50*	*48*
- senile atrophy	47 (2.4)	45 (2.6)	48 (2.5)	49 (2.8)	48 (3.1)
Adrenals					
No. examined	*50*	*50*	*49*	*50*	*49*
- cortical atrophy	1 (2.0)	3 (2.0)	5 (1.6)	9*** (1.4)	3 (2.0)
- focal fatty change	3 (1.0)	6 (1.3)	6 (1.3)	8 (1.0)	11* (1.4)
- diffuse hypertrophy	0 (—)	0 (—)	2 (2.0)	3 (2.0)	4 (2.0)

From Blumbach (2006b)
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 40. Incidences of thyroid neoplasms: all rats

Organ/lesion	Incidence[a]				
	Dose (mg/kg bw per day)				
	0	9	27	83	250
Males					
No. of animals examined	*50*	*50*	*48*	*49*	*49*
Thyroid					
- follicular adenoma	3	1	6	2	9
- follicular carcinoma	2	1	0	0	3
- combined adenoma/carcinoma	5	2	6	2	10
Females					
No. of animals examined	*50*	*50*	*49*	*50*	*48*
Thyroid					
- follicular adenoma	3	1	2	0	0
- follicular carcinoma	0	0	1	0	1

From Blumbach (2006b)
[a] Historical controls: thyroid follicular adenoma 3.29% (range 0.00–14.29%, derived from 40 studies on 2741 animals during the period February 1982 – June 2004).

There was an increased incidence of thyroid follicular adenomas in males at 250 mg/kg bw per day. The incidence of thyroid follicular adenoma in this group exceeded the control incidence and slightly exceeded the historical control range. The incidences of adenoma in male groups treated at lower dose levels and in all female groups were comparable to those in controls. There was no effect on the incidences of follicular carcinoma in either sex at any dose level (Table 40).

The NOAEL was 27 mg/kg bw per day, based on the occurrence of reduced body weight gain in females and hepatic periportal fatty degeneration in males at 83 mg/kg bw per day. Histopathological alterations in the liver, kidneys, thyroid, ovaries and adrenals were observed at 250 mg/kg bw per day (Blumbach, 2006b).

Table 41. Genotoxicity studies with penthiopyrad

End-point	Test object	Concentration	Purity (%)	Results	Reference
In vitro					
Reverse mutation	*Salmonella typhimurium* and *Escherichia coli*	0, 4.69, 9.38, 18.8, 37.5, 75, 150, 300, 600 or 1200 µg/plate (+S9) 0, 2.34, 4.69, 9.38, 18.8, 37.5, 75, 150, 300, 600 or 1200 µg/plate (−S9)	99.8	Negative	Nakajima (2000a)
DNA repair	*Bacillus subtilis*	0, 88.5, 177, 354, 708, 1416, 2831, 5663 or 11 325 µg/disc (+S9) 0, 177, 354, 708, 1416, 2831, 5663, 11 325 or 22 650 µg/disc (−S9)	99.8	Negative	Nakajima (2000b)
Mammalian cell gene mutation	Mouse lymphoma L5178Y cells	0, 4.32, 6.18, 8.82, 12.6, 18.0, 25.7, 36.8 or 52.5 µg/ml (+S9) 0, 6.18, 8.82, 12.6, 18.0, 25.7, 36.8, 52.5 or 75.0 µg/ml (−S9)	99.8	Negative	Masumori (2000a)
Chromosomal aberration	Chinese hamster lung cells	0, 81.9, 102, 128, 160, 200 or 250 µg/ml (+S9, 6 h) 0, 52.4, 65.5, 81.9, 102, 128, 160 or 200 µg/ml (−S9, 6 h) 0, 52.4, 65.5, 81.9, 102, 128 or 160 µg/ml (−S9, 24 h)	99.8	Negative	Nakajima (2000c)
In vivo					
Mouse micronucleus	BDF1 mice, male	0, 500, 1000 or 2000 mg/kg bw[a] Sampling time: 24 h	99.8	Negative	Nakajima (2000d)
Unscheduled DNA synthesis	CD rats, male	0, 1000 or 2000 mg/kg bw Sampling times: 2 h, 16 h	99.8	Negative	Masumori (2000b)

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

[a] Given as two doses of 250, 500 or 1000 mg/kg bw 24 hours apart.

2.4 Genotoxicity

A battery of GLP-compliant studies of mutagenicity with penthiopyrad was conducted to assess its potential for inducing gene mutation, chromosomal aberration and unscheduled deoxyribonucleic acid (DNA) synthesis. The study results (summarized in Table 41) were negative. Overall, penthiopyrad did not demonstrate any genotoxic potential.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

In a non-guideline reproduction range-finding study, penthiopyrad (purity 98.8%) was administered continuously in the diet to eight Wistar rats of each sex per group at a dietary concentration of 0, 680, 1360, 3400 or 8500 ppm (actual doses shown in Table 42). Parental animals were given test article diet formulations for 3 weeks prior to mating. On postnatal day (PND) 21, all pups and their respective dams were sacrificed.

Table 42. Penthiopyrad doses during the different phases of a range-finding one-generation study in rats

Phase of the study	Dose (mg/kg bw per day)			
	Dietary concentration (ppm)			
	680	1360	3400	8500
Males				
Premating	44.6	88.5	220	555
Breeding	34.5–37.9	65.6–73.2	171–187	438–477
Females				
Premating	52.1	103.5	240	604
Gestation	86.5	157.7	417	941
Lactation	75.0	139.6	358	829

From Teramoto (2004)

When compared with concurrent controls, no treatment-related changes were observed in the following parameters: mortality, clinical signs and reproductive function and performance in the parental animals; and mean number of pups delivered, survival indices, sex ratios, clinical signs and gross pathology in the offspring. In parental animals at 3400 ppm and above, feed consumption was reduced and absolute and relative (to body weight) liver weights were increased in the males and females. Absolute and relative (to body weight) thyroid weights were also increased in males. Additionally at 8500 ppm, body weight and body weight gain and absolute and relative (to body weight) spleen (females only) weights were reduced, and the liver of one female was dark in colour. No treatment-related findings were noted at 680 or 1360 ppm. In the offspring at 8500 ppm, decreased body weights were noted in both sexes from lactation days (LDs) 4 to 21, and absolute and relative (to body weight) thymus and spleen weights were reduced.

The NOAEL for parental toxicity was 1360 ppm, based on decreased feed consumption and increased liver and thyroid weights at 3400 ppm. The NOAEL for reproductive effects was 8500 ppm, the highest dose tested. The NOAEL for offspring toxicity was 3400 ppm, based on decreased body weight and decreased thymus and spleen weights at 8500 ppm (Teramoto, 2004).

In a two-generation reproductive toxicity study in rats, penthiopyrad (purity 98.8%) was administered in the diet to 24 Wistar rats of each sex per dose at concentrations of 0, 200, 1000 or 5000 ppm for 10 weeks prior to mating and through lactation until weaning of the F_1 offspring. Groups of 24 male and 24 female F_1 generation offspring were then similarly treated. Overall achieved dose levels were within the ranges 11.0–19.0, 54.0–95.6 and 278–480 mg/kg bw per day (Tables 43 and 44). 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

Table 43. Penthiopyrad doses for male rats during the different phases of a two-generation study

Dietary concentration (ppm)	Generation	Dose (mg/kg bw per day)	
		Premating period	Breeding period
200	F_0	12.3	8.38–17.8
	F_1	15.1	8.62–20.9
1000	F_0	60.5	41.5–87.8
	F_1	76.1	42.1–107.5
5000	F_0	311	215–451
	F_1	403	227–588

From Teramoto (2005)

Table 44. Penthiopyrad doses for female rats during the different phases of a two-generation study

Dietary concentration (ppm)	Generation	Dose (mg/kg bw per day)		
		Premating period	Gestation period	Lactation period
200	F_0	15.3	12.0–13.8	23.0–41.8
	F_1	17.3	11.4–13.1	23.0–40.2
1000	F_0	75.9	61.1–70.4	118.0–208.4
	F_1	86.3	57.0–64.3	118.6–205.8
5000	F_0	372	305–335	582–966
	F_1	437	292–325	618–980

From Teramoto (2005)

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.

Two F_0 generation females at 5000 ppm died prematurely, but neither death was considered to be treatment related. There were no treatment-related clinical signs in either generation at any dose level. Treatment-related decreases in body weight gain occurred in F_1 males and females at 5000 ppm and in F_1 males at 1000 ppm, but not in F_0 generation animals (Tables 45 and 46). There were no treatment-related effects on feed consumption in either generation at any dose level. The mean age at completion of preputial separation or vaginal patency in F_1 parental animals was slightly prolonged at 5000 ppm, but the mean body weight at completion in both sexes was comparable to the control value, suggesting that the slight delay was due to low body weight at 5000 ppm (Table 47). The estrous cyclicity in both generations at all dose levels was unaffected by treatment. All treated males and females of both generations at all dose levels successfully mated, and the mean time to mating was unaffected by treatment at all dose levels. Female fertility and gestation indices were also unaffected by treatment at all dose levels, and there was no effect of treatment on the duration of gestation. The mean numbers of implantation sites in all treated groups of both generations were comparable to the control group values. Male fertility was also unaffected by treatment at all dose

Table 45. Group mean body weights and body weight gains in rats at selected intervals during pre-mating

Generation and sex	Dietary concentration (ppm)	Group mean body weight (g) ± SD					Body weight gain (g) ± SD
		Pre-mating week					
		0	2	4	8	10	Weeks 0–10
F_0 males	0	152 ± 5	243 ± 9	306 ± 14	378 ± 26	398 ± 29	246 ± 28
	200	152 ± 5	240 ± 11	304 ± 19	376 ± 30	397 ± 33	245 ± 30
	1000	152 ± 5	243 ± 8	305 ± 16	372 ± 27	388 ± 29	236 ± 28
	5000	152 ± 5	238 ± 9	301 ± 17	378 ± 29	398 ± 32	246 ± 31
F_1 males	0	64 ± 5	155 ± 12	247 ± 17	372 ± 32	405 ± 37	341 ± 34
	200	64 ± 5	153 ± 8	242 ± 14	364 ± 26	393 ± 29	329 ± 29
	1000	64 ± 4	153 ± 10	240 ± 15	355 ± 27	383 ± 30*	319 ± 29*
	5000	61 ± 8	142 ± 12**	230 ± 18**	345 ± 24**	375 ± 27**	314 ± 25**
F_0 females	0	117 ± 4	160 ± 7	188 ± 10	219 ± 13	229 ± 13	112 ± 11
	200	117 ± 4	157 ± 7	188 ± 9	219 ± 11	227 ± 12	110 ± 12
	1000	117 ± 4	157 ± 7	186 ± 10	217 ± 11	225 ± 11	108 ± 11
	5000	117 ± 4	157 ± 9	182 ± 9	213 ± 11	222 ± 13	105 ± 11
F_1 females	0	60 ± 6	124 ± 8	164 ± 12	215 ± 18	232 ± 19	172 ± 18
	200	61 ± 4	124 ± 7	164 ± 12	214 ± 15	230 ± 15	170 ± 15
	1000	60 ± 3	123 ± 5	162 ± 9	212 ± 15	226 ± 13	165 ± 13
	5000	58 ± 6	119 ± 8	159 ± 9	206 ± 12	220 ± 11*	162 ± 10

From Teramoto (2005)
SD, standard deviation; * $P < 0.05$; ** $P < 0.01$

Table 46. Group mean body weights during gestation and lactation in rats

Dietary concentration (ppm)	Group mean body weight (g) ± standard deviation							
	Gestation day				Lactation day			
	0	7	14	20	0	7	14	20
F_0 generation[a]								
0	233 ± 15	255 ± 16	279 ± 18	337 ± 21	261 ± 20	282 ± 16	294 ± 16	280 ± 17
200	232 ± 12	252 ± 12	276 ± 12	333 ± 16	258 ± 12	275 ± 13	287 ± 12	271 ± 11
1000	231 ± 14	252 ± 13	277 ± 15	337 ± 16	254 ± 19	278 ± 15	290 ± 16	275 ± 14
5000	225 ± 13	246 ± 11	270 ± 12	329 ± 16	247 ± 15*	274 ± 13	285 ± 14	276 ± 11
F_1 generation[b]								
0	237 ± 21	252 ± 20	275 ± 22	332 ± 23	255 ± 21	283 ± 21	296 ± 23	279 ± 22
200	235 ± 15	252 ± 15	275 ± 17	331 ± 23	258 ± 18	284 ± 17	293 ± 16	275 ± 13
1000	231 ± 14	249 ± 15	272 ± 16	330 ± 18	255 ± 19	280 ± 18	294 ± 18	278 ± 17
5000	226 ± 12	244 ± 14	269 ± 16	331 ± 21	251 ± 20	280 ± 21	294 ± 21	282 ± 18

From Teramoto (2005)
* $P \leq 0.05$
[a] F_0 generation: 0 ppm, $n = 21$; 200 ppm, $n = 24$; 1000 ppm, $n = 21$; 5000 ppm, $n = 21$–22.
[b] F_1 generation: 0 ppm, $n = 23$; 200 ppm, $n = 23$; 1000 ppm, $n = 22$; 5000 ppm, $n = 23$.

Table 47. Sexual development in F_1 parental rats

Dietary concentration (ppm)	Completion of preputial separation		Completion of vaginal opening	
	Mean age (days)	Mean weight (g)	Mean age (days)	Mean weight (g)
0	40.7 ± 1.5[a]	179 ± 16	30.8 ± 2.4	97 ± 12
200	41.0 ± 2.0	177 ± 16	30.5 ± 1.8	95 ± 11
1000	41.5 ± 2.1	180 ± 17	30.4 ± 2.1	95 ± 12
5000	42.6 ± 1.7**	174 ± 11	32.1 ± 2.6	98 ± 12

From Teramoto (2005)
** $P < 0.01$
[a] ± standard deviation.

Table 48. Incidence of treatment-related gross pathological alterations in F_0 and F_1 parental rats

Generation/sex	Organ/alteration	Incidence			
		Dietary concentration (ppm)			
		0	200	1000	5000
F_0 male	*Thyroid: no. examined*	*24*	*24*	*24*	*24*
	- enlargement	2	3	3	8*
F_1 male	*Thyroid: no. examined*	*24*	*24*	*24*	*24*
	- enlargement	3	1	4	7
F_0 female	*Thyroid: no. examined*	*24*	*24*	*24*	*24*
	- enlargement	2	0	2	6
	Liver: no. examined	*24*	*24*	*24*	*24*
	- dark in colour	0	0	0	15***
F_1 female	*Thyroid: no. examined*	*24*	*24*	*24*	*24*
	- enlargement	1	2	3	5
	Liver: no examined	*24*	*24*	*24*	*24*
	- dark in colour	0	0	0	16***

From Teramoto (2005)
* $P < 0.05$; *** $P < 0.001$

levels, and there was no effect of treatment on testicular sperm head count, epididymal sperm count, sperm motility or sperm morphology at any dose level in either generation.

Treatment-related gross pathology alterations comprised dark liver colour in females of both generations at 5000 ppm and increased incidences of thyroid enlargement in both sexes treated at 5000 ppm of both generations (Table 48). Treatment-related effects on organ weights occurred in the liver, adrenals and thyroid. Absolute and/or relative liver weights were increased in both sexes of both generations at 5000 ppm and in females of both generations at 1000 ppm. Absolute and/or relative adrenal and thyroid weights were increased in both sexes of both generations at 5000 ppm, and adrenal weight was also increased in F_1 females at 1000 ppm (Table 49). There were no treatment-related histopathological alterations in the reproductive organs and pituitary gland at 5000 ppm. Treatment-related hypertrophic alterations occurred in the liver, adrenals and thyroid, which could be correlated with increases in the organ weights. Female F_1 animals at 1000 ppm also showed an increased incidence of adrenal cortical hypertrophy (Table 50). There was no effect of treatment at 5000 ppm on the F_1 female group mean ovarian follicle count.

Table 49. Treatment-related organ weight changes in F_0 and F_1 parental rats

Generation/sex	Dietary concentration (ppm)	Body weight[a] (g)	Type of organ weight	Group mean weight		
				Liver	Adrenals	Thyroid
F_0 male	0	448 ± 34	Absolute (g)	13.3	0.030	0.029
			Relative (%)	2.96	0.006 74	0.006 34
	200	443 ± 35	Absolute (g)	13.2	0.030	0.029
			Relative (%)	2.98	0.006 70	0.006 57
	1000	436 ± 31	Absolute (g)	13.6	0.003 0	0.033
			Relative (%)	3.10	0.006 77	0.007 64
	5000	448 ± 40	Absolute (g)	15.8**	0.033*	0.040**
			Relative (%)	3.54**	0.074 0	0.009 14*
F_1 male	0	466 ± 42	Absolute (g)	14.6	0.032	0.032
			Relative (%)	3.12	0.006 85	0.006 93
	200	450 ± 33	Absolute (g)	13.8	0.032	0.029
			Relative (%)	3.07	0.007 13	0.006 42
	1000	442 ± 37	Absolute (g)	13.9	0.032	0.035
			Relative (%)	3.15	0.007 18	0.007 84
	5000	432 ± 34**	Absolute (g)	15.5	0.034	0.038
			Relative (%)	3.59**	0.007 97**	0.008 90
F_0 female	0	259 ± 16	Absolute (g)	9.5	0.040	0.023
			Relative (%)	3.66	0.015 4	0.008 73
	200	256 ± 12	Absolute (g)	9.5	0.040	0.021
			Relative (%)	3.70	0.015 6	0.008 22
	1000	259 ± 15	Absolute (g)	10.1	0.040	0.023
			Relative (%)	3.89*	0.015 5	0.008 91
	5000	252 ± 11	Absolute (g)	11.3**	0.046**	0.032**
			Relative (%)	4.46**	0.018 4**	0.012 77**
F_1 female	0	267 ± 22	Absolute (g)	10.2	0.040	0.021
			Relative (%)	3.83	0.015 0	0.008 02
	200	265 ± 16	Absolute (g)	10.0	0.040	0.022
			Relative (%)	3.76	0.014 8	0.008 42
	1000	261 ± 15	Absolute (g)	11.0	0.043	0.025
			Relative (%)	4.20**	0.016 6*	0.009 40
	5000	258 ± 15	Absolute (g)	12.0**	0.050**	0.030**
			Relative (%)	4.66**	0.019 3**	0.011 74**

From Teramoto (2005)
* $P < 0.05$; ** $P < 0.01$
[a] ± standard deviation.

Table 50. Incidence of treatment-related histopathological alterations in F_0 and F_1 parental rats

Generation/sex	Organ/alteration	Incidence			
		Dietary concentration (ppm)			
		0	200	1000	5000
F_0 male	*Thyroid: no. examined*	*24*	*24*	*24*	*24*
	- follicular hypertrophy	0	0	1	11***
	Adrenal: no. examined	*24*	*24*	*24*	*24*
	- cortical hypertrophy	1	3	0	5
	Liver: no. examined	*24*	*24*	*24*	*24*
	- hepatocyte hypertrophy	0	0	1	9**
F_1 male	*Thyroid: no. examined*	*24*	*24*	*24*	*24*
	- follicular hypertrophy	0	1	0	8**
	Adrenal: no. examined	*24*	*24*	*24*	*24*
	- cortical hypertrophy	2	4	2	6
	Liver: no. examined	*24*	*24*	*24*	*24*
	- hepatocyte hypertrophy	0	0	0	7**
F_0 female	*Thyroid: no. examined*	*24*	*24*	*24*	*24*
	- follicular hypertrophy	0	0	2	11***
	Adrenal: no. examined	*24*	*24*	*24*	*24*
	- cortical hypertrophy	2	0	2	20***
	Liver: no. examined	*24*	*24*	*24*	*24*
	- hepatocyte hypertrophy	0	0	0	11***
F_1 female	*Thyroid: no. examined*	*24*	*24*	*24*	*24*
	- follicular hypertrophy	0	1	2	11***
	Adrenal: no. examined	*24*	*24*	*24*	*24*
	- cortical hypertrophy	3	1	10*	23***
	Liver: no. examined	*24*	*24*	*24*	*24*
	- hepatocyte hypertrophy	0	0	3	14***

From Teramoto (2005)
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

The mean number of pups delivered and the sex ratio were unaffected by treatment at all dose levels in both generations. There were no treatment-related clinical signs or increased pup mortality during the lactation period at any dose level in either generation. There was no effect of treatment on the group mean pup weight at birth at any dose level in either generation (Table 51). However, F_1 males at 5000 ppm showed reduced body weight gain from day 4, and F_2 males and females showed reduced weight gain from day 14 of lactation. Other than small body size, related to reduced pre-weaning body weight gain, in some F_1 and F_2 pups at 5000 ppm, there were no treatment-related gross pathological findings in F_1 and F_2 weanlings at any dose level. Reduced absolute and/or relative thymus weights occurred in F_1 and F_2 males and F_2 females at 5000 ppm, and reduced absolute and relative spleen weights occurred in F_1 and F_2 males and F_1 females at 5000 ppm. Relative brain weights were significantly increased in F_1 and F_2 males at 5000 ppm and in F_2 females at 1000 and 5000 ppm. These differences in relative brain weight were considered to reflect reduced body weight at the two highest dose levels. No treatment-related histopathological alterations were evident in the spleen and thymus of F_1 and F_2 weanlings in the 5000 ppm group (Table 52).

Table 51. Group mean body weight during lactation of F_1 and F_2 generation rat offspring[a]

Generation/ sex	Dietary concentration (ppm)	Group mean body weight (g) ± standard deviation				
		Lactation day				
		0	4	7	14	21
F_1 male	0	6.2 ± 0.6	11.1 ± 1.4	17.7 ± 1.5	35.6 ± 2.6	56.7 ± 4.3
	200	6.1 ± 0.4	11.0 ± 1.0	17.5 ± 1.3	34.9 ± 2.6	55.4 ± 4.1
	1000	6.1 ± 0.6	11.1 ± 1.3	17.8 ± 1.7	35.2 ± 2.3	55.0 ± 3.7
	5000	5.9 ± 0.5	10.2 ± 1.2*	16.3 ± 1.6*	31.8 ± 31.8**	49.0 ± 4.8**
F_2 male	0	6.0 ± 0.6	10.8 ± 1.4	17.4 ± 2.0	35.5 ± 2.8	56.8 ± 4.8
	200	6.1 ± 0.6	11.2 ± 1.1	18.0 ± 1.6	35.9 ± 2.7	55.7 ± 4.5
	1000	6.0 ± 0.5	11.2 ± 1.2	17.7 ± 1.3	35.1 ± 2.3	55.3 ± 3.0
	5000	6.1 ± 0.4	10.7 ± 0.9	16.9 ± 1.4	33.5 ± 2.9*	51.5 ± 3.6**
F_1 female	0	5.9 ± 0.5	10.7 ± 1.2	17.1 ± 1.4	34.5 ± 2.6	54.6 ± 3.9
	200	5.8 ± 0.5	10.6 ± 1.2	17.0 ± 1.5	34.1 ± 2.7	53.6 ± 4.1
	1000	5.7 ± 0.5	10.6 ± 1.1	16.9 ± 1.7	33.7 ± 2.3	52.0 ± 3.6
	5000	5.7 ± 0.5	10.0 ± 1.2	16.2 ± 1.5	31.5 ± 2.5**	48.2 ± 4.4**
F_2 female	0	5.7 ± 0.5	10.3 ± 1.2	16.6 ± 1.7	34.4 ± 2.4	54.0 ± 4.1
	200	5.8 ± 0.5	10.8 ± 1.1	17.4 ± 1.6	34.9 ± 2.4	53.8 ± 3.3
	1000	5.7 ± 0.5	10.7 ± 1.2	17.0 ± 1.4	33.9 ± 2.3	52.6 ± 3.0
	5000	5.8 ± 0.4	10.4 ± 0.8	16.2 ± 1.2	32.3 ± 2.3**	49.2 ± 2.8**

From Teramoto (2005)
* $P < 0.05$; ** $P < 0.01$
[a] $n = 21–23$.

Table 52. Selected group mean organ weights in F_1 and F_2 weanling rats at 25–27 days of age

Generation/sex	Dietary concentration (ppm)	Body weight (g)	Type of organ weight	Group mean weight (g) ± standard deviation		
				Brain	Thymus	Spleen
F_1 male	0	83 ± 8	Absolute (g)	1.55 ± 0.057	0.30 ± 0.057	0.32 ± 0.042
			Relative (%)	1.88 ± 0.15	0.36 ± 0.056	0.39 ± 0.047
	200	80 ± 7	Absolute (g)	1.54 ± 0.044	0.30 ± 0.042	0.33 ± 0.063
			Relative (%)	1.94 ± 0.16	0.37 ± 0.050	0.41 ± 0.065
	1000	80 ± 7	Absolute (g)	1.55 ± 0.047	0.29 ± 0.041	0.31 ± 0.053
			Relative (%)	1.94 ± 0.15	0.36 ± 0.047	0.39 ± 0.058
	5000	75 ± 7**	Absolute (g)	1.53 ± 0.065	0.25 ± 0.053**	0.26 ± 0.042**
			Relative (%)	2.04 ± 0.16**	0.33 ± 0.045	0.34 ± 0.042*
F_2 male	0	77 ± 7	Absolute (g)	1.52 ± 0.060	0.27 ± 0.039	0.32 ± 0.041
			Relative (%)	1.99 ± 0.15	0.34 ± 0.041	0.41 ± 0.046
	200	75 ± 6	Absolute (g)	1.54 ± 0.052	0.25 ± 0.036	0.29 ± 0.047
			Relative (%)	2.07 ± 0.16	0.33 ± 0.045	0.39 ± 0.057
	1000	75 ± 4	Absolute (g)	1.53 ± 0.041	0.26 ± 0.031	0.29 ± 0.029
			Relative (%)	2.05 ± 0.11	0.35 ± 0.045	0.39 ± 0.058
	5000	70 ± 5**	Absolute (g)	1.52 ± 0.045	0.22 ± 0.028**	0.26 ± 0.038**
			Relative (%)	2.17 ± 0.13**	0.31 ± 0.042*	0.37 ± 0.046**

Table 52 (continued)

Generation/sex	Dietary concentration (ppm)	Body weight (g)	Type of organ weight	Group mean weight (g) ± standard deviation		
				Brain	Thymus	Spleen
F_1 female	0	76 ± 7	Absolute (g)	1.47 ± 0.047	0.28 ± 0.051	0.28 ± 0.051
			Relative (%)	1.95 ± 0.14	0.37 ± 0.054	0.37 ± 0.076
	200	76 ± 7	Absolute (g)	1.48 ± 0.053	0.29 ± 0.047	0.27 ± 0.033
			Relative (%)	1.98 ± 0.19	0.38 ± 0.042	0.36 ± 0.038
	1000	72 ± 7	Absolute (g)	1.48 ± 0.068	0.26 ± 0.046	0.26 ± 0.033
			Relative (%)	2.06 ± 0.18	0.35 ± 0.059	0.36 ± 0.050
	5000	71 ± 6	Absolute (g)	1.45 ± 0.045	0.26 ± 0.047	0.23 ± 0.031**
			Relative (%)	2.06 ± 0.014	0.36 ± 0.046	0.33 ± 0.036*
F_2 female	0	70 ± 5	Absolute (g)	1.45 ± 0.053	0.26 ± 0.035	0.28 ± 0.050
			Relative (%)	2.07 ± 0.14	0.37 ± 0.042	0.39 ± 0.072
	200	70 ± 5	Absolute (g)	1.47 ± 0.054	0.25 ± 0.038	0.26 ± 0.025
			Relative (%)	2.10 ± 0.12	0.35 ± 0.054	0.37 ± 0.033
	1000	68 ± 4	Absolute (g)	1.47 ± 0.056	0.25 ± 0.032	0.25 ± 0.031
			Relative (%)	2.16 ± 0.11*	0.36 ± 0.048	0.37 ± 0.048
	5000	67 ± 3*	Absolute (g)	1.46 ± 0.037	0.22 ± 0.031**	0.25 ± 0.034
			Relative (%)	2.18 ± 0.10**	0.33 ± 0.043**	0.37 ± 0.043

From Teramoto (2005)
* $P < 0.05$; ** $P < 0.01$

The parental NOAEL was 200 ppm (11.0 mg/kg bw per day), based on reduced body weight gain in F_1 adult males, increased liver weight in F_0 and F_1 adult females and increased relative adrenal weight with increased incidence of cortical hypertrophy in F_1 females at 1000 ppm (54.0 mg/kg bw per day). The reproductive toxicity NOAEL was 5000 ppm (278 mg/kg bw per day), the highest dose tested. The offspring NOAEL was 1000 ppm (54.0 mg/kg bw per day), based on reduced body weight and body weight gain, delay in preputial separation and a statistically significant decrease in absolute thymus weights at 5000 ppm (278 mg/kg bw per day) (Teramoto, 2005).

(b) Developmental toxicity

Rats

In a preliminary study of prenatal developmental toxicity, groups of six mated Wistar female rats were given penthiopyrad (purity 98.6%) suspended in a solution of 0.5% aqueous carboxymethylcellulose/0.1% Tween 80 by oral gavage at a dose of 0, 250, 500 or 1000 mg/kg bw per day from gestation day (GD) 6 to GD 19. All dams were sacrificed on GD 20, and the uterine contents were examined.

There was no evidence of maternal or developmental toxicity, and caesarean sectioning at day 20 also revealed no treatment-related changes in ovaries or uteri.

The NOAEL for maternal and developmental toxicity was 1000 mg/kg bw per day, the highest dose tested (Pattern, 2005).

In a developmental toxicity study, penthiopyrad (purity 98.6%) was administered to mated female Wistar Hanover (HsdBrl) rats (22 per dose) via gavage from day 6 to day 19 of gestation at a dose level of 0, 62.5, 250 or 1000 mg/kg bw per day at 10 ml/kg bw in aqueous carboxymethylcellulose (0.5% weight per volume [w/v])/Tween 80 (0.1% w/v). The animals were sacrificed on day 20 after

Table 53. Selected group mean body weights and body weight gains in rats during gestation

Dose (mg/kg bw per day)	Mean body weight (g) ± SD	Group mean body weight gain (g) ± SD					Mean weight (g) ± SD
	Day(s)						
	0	6	0–6	6–9	6–12	6–18	6–20	20
0	206 ± 9	227 ± 10	21 ± 5	8 ± 3	21 ± 4	66 ± 7	89 ± 10	316 ± 16
62.5	206 ± 8	228 ± 10	22 ± 4	8 ± 3	21 ± 4	65 ± 9	89 ± 12	317 ± 17
250	205 ± 9	227 ± 8	21 ± 6	6 ± 5	19 ± 6	61 ± 7	84 ± 11	310 ± 14
1000	203 ± 10	224 ± 12	20 ± 4	5 ± 4*	20 ± 4	61 ± 10	83 ± 16	306 ± 24

From Pattern (2006a)
SD, standard deviation; * $P < 0.05$

Table 54. Group mean rat litter data

Dose (mg/kg bw per day)	CL	Implantations	Resorptions			Live young			Sex ratio (% M)	Implantation loss (%)	
			Early	Late	Total	Male	Female	Total		Pre-	Post-
0	13.3 ± 1.8	12.1 ± 2.0	0.5	0.0	0.5	5.4 ± 2.1	6.2 ± 1.9	11.6 ± 2.0	46.3	10.5	4.0
62.5	13.8 ± 1.7	12.4 ± 2.3	0.8	0.0	0.8	5.7 ± 2.3	5.8 ± 2.2	11.5 ± 2.5	49.3	11.1	7.0
250	12.5 ± 1.7	11.3 ± 2.1	0.5	0.0	0.5	4.9 ± 1.8	5.9 ± 1.7	10.8 ± 2.2	45.1	9.2	5.3
1000	13.1 ± 2.0	11.7 ± 2.2	1.4*	0.0	1.5*	5.3 ± 2.5	4.9 ± 2.4*	10.2 ± 2.9	50.7	10.8	13.0**

From Pattern (2006a)
CL, corpora lutea; M, male; * $P < 0.05$; ** $P < 0.01$

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 adverse clinical signs that could be attributed to treatment with penthiopyrad at any dose level. Approximately 50% of females at 250 mg/kg bw per day and all females at 1000 mg/kg bw per day showed increased salivation in response to dose administration. However, the effect was transient, and similar effects were not observed in a preliminary study (same regimen) at doses up to 1000 mg/kg bw per day. At 1000 mg/kg bw per day, body weight gain and feed consumption were marginally reduced following the onset of treatment (days 6–9 after mating), but subsequently weight gain and feed consumption were similar to those of the controls. The group mean body weight on day 20 was not significantly different from that of the controls. Body weight gain, feed consumption and group mean body weight on day 20 at 62.5 or 250 mg/kg bw per day were unaffected by treatment (Table 53). At 1000 mg/kg bw per day, the gravid uterine weight was significantly lower than that of the controls, but the overall maternal weight gain after adjustment for the gravid uterine weight was unaffected by treatment. Gravid uterine weight and adjusted maternal weight gain were unaffected by treatment at 62.5 or 250 mg/kg bw per day. On day 20 of gestation, macroscopic examination of females at necropsy revealed no findings that could be associated with treatment at any dose level. All treated and control females had a live litter at necropsy on day 20 of gestation, with the exception of one non-pregnant female at 1000 mg/kg bw per day. At 1000 mg/kg bw per day, there was a significant increase in the number of early resorptions and resultant post-implantation loss, and the live litter size was marginally less than that of the controls. At 62.5 and 250 mg/kg bw per day, litter data, as assessed by the numbers of corpora lutea, implantations, resorptions and live young, pre-implantation and post-implantation losses and sex ratio, were unaffected by treatment (Table 54). Placental and fetal weights were unaffected by treatment at all dose levels, but litter weight at 1000 mg/kg bw per day was lower than that of the controls

Table 55. Group mean placental, fetal and litter weights in rats

Dose (mg/kg bw per day)	Group mean values ± standard deviation					
	Placental weight (g)	Number of live young	Litter weight (g)	Fetal weight (g)		
				Males	Females	Overall
0	0.52 ± 0.05	11.6 ± 2.0	40.18 ± 6.92	3.59 ± 0.19	3.39 ± 0.15	3.47 ± 0.15
62.5	0.50 ± 0.04	11.5 ± 2.5	38.99 ± 7.93	3.49 ± 0.28	3.29 ± 0.29	3.40 ± 0.29
250	0.52 ± 0.06	10.8 ± 2.2	37.25 ± 7.52	3.56 ± 0.23	3.38 ± 0.14	3.47 ± 0.14
1000	0.51 ± 0.05	10.2 ± 2.9	35.34 ± 10.73	3.56 ± 0.22	3.35 ± 0.20	3.47 ± 0.20

From Pattern (2006a)

Table 56. Group incidences of major abnormalities in rats

Abnormality	Fetal incidence				Litter incidence			
	Dose (mg/kg bw per day)							
	0	62.5	250	1000	0	62.5	250	1000
Number examined	*255*	*254*	*237*	*214*	*22*	*22*	*22*	*21*
Number affected	*5*	*11*	*4*	*0*	*4*	*6*	*2*	*0*
Small/misshapen pituitary	0	2	0	0	0	0	1	0
Fused maxilla to jugal	0	0	2	0	0	0	1	0
Cleft palate; shortened upper/lower jaw; misshapen/distorted rib cage; distorted cervical/ lower lumbar vertebrae; cervical/lumbar lordosis; bent ilium; malpositioned ischium; bent scapula and/or radium/ulna	0	0	2	0	0	0	1	0
Bent scapula	[a]	[b]	0	0	3	4	0	0
Short and/or thickened and/or bent humerus	[a]	[b]	0	0	3	5	0	0
Short/bent and misshapen radius	0	1	0	0	0	1	0	0

From Pattern (2006a)

[a] Two fetuses with bent scapula; one with short/thickened/bent humerus; two with bent scapula and short/thickened/bent right humerus.

[b] One fetus with bent right scapula; one with short/bent right humerus; one with short/bent and misshapen bilateral humerus, six with bent scapula and short/thickened/bent humerus.

by 12.0% as a consequence of lower litter size. Litter weights at 62.5 and 250 mg/kg bw per day were unaffected by treatment (Table 55). There was no increased incidence of malformations (Tables 56–58); however, there was an increase in partially undescended thymus at the highest dose tested (Table 59) that exceeded both the concurrent and historical control range (Table 60).

The maternal NOAEL was 250 mg/kg bw per day, based on minimal transient reduced body weight gain, feed consumption, resorptions and post-implantation survival at 1000 mg/kg bw per day. The fetal NOAEL was 250 mg/kg bw per day, based on resorptions and a slight decrease in post-implantation survival, litter size and gravid uterine weight at 1000 mg/kg bw per day (Pattern, 2006a). The resorptions and resultant post-implantation loss are considered possibly due to maternal and/or fetal toxicity, and therefore these effects were included in both the maternal and fetal lowest-observed-adverse-effect levels (LOAELs).

Table 57. Selected historical control data in rats

	Study No.[a]				
	1	2	3	4	5
No. of fetuses (litters) examined	*109 (20)*	*125 (22)*	*121 (22)*	*113 (21)*	*46 (8)*
Major skeletal abnormalities					
- bent/thickened/short scapula	1 (1)	1 (1)	0	1 (1)	1 (1)
- bent/short/thickened humerus	0	0	0	2 (2)	0
Minor skeletal abnormalities					
- partially fused/bridge of ossification maxilla to jugal	1 (1)	4 (4)	16 (10)	15 (7)	0
- medially thickened/kinked ribs	5 (4)	5 (4)	19 (8)	13 (9)	5 (3)
Minor visceral abnormalities					
- thymus partially undescended	0	1 (1)	0	0	1 (1)
- diaphragm thin with protruding liver	2 (2)	10 (8)	5 (3)	1 (1)	0
- testis(es) displaced	5 (5)	6 (6)	5 (4)	2 (2)	0

From Pattern (2006a)

[a] Regarding historical control data, there were five carboxymethylcellulose vehicle gavage studies (2002–2005).

Table 58. Group incidences of minor skeletal abnormalities/variants in rats

	Fetal incidence[a]				Litter incidence			
	Dose (mg/kg bw per day)							
	0	62.5	250	1000	0	62.5	250	1000
Number examined	*123*	*119*	*118*	*106*	*22*	*22*	*22*	*21*
Cranial								
- sutural bone	1	—	1	—	1	—	1	—
- partially fused/bridge of ossification maxilla to jugal	14	5	18	16	8	5	9	11
Ribs								
- medially thickened/kinked	17	18	4	2*	8	12	3	2
Sternebrae								
- offset alignment	2	3	2	6a	2	3	2	5
- bipartite ossified/misshapen	—	—	—	2a	—	—	—	2
- partially fused	—	—	—	1b	—	—	—	1
- additional	—	—	—	1b	—	—	—	1
Costal cartilage								
- offset alignment	—	—	—	2a	—	—	—	2
- 2nd not connected to sternum	—	—	—	1b	—	—	—	1
- additional	—	—	—	2a	—	—	—	1
- partially fused	—	—	—	1a	—	—	—	1
Appendicular								
- bent scapula, minimal	—	—	—	1	—	—	—	1
Total affected by one or more of the above	34	23	22	24	15	16	11	14
Rib and vertebral configuration								
- cervical rib	10	12	7	12	7	10	4	8
- number with 13/14 or 14/14 ribs	29	44	43	35	13	18	18	15

Table 58 (continued)

	Fetal incidence[a]				Litter incidence			
	Dose (mg/kg bw per day)							
	0	62.5	250	1000	0	62.5	250	1000
- complete 14th rib(s)	2	—	1	2	2	—	1	2
- 20 thoracolumbar vertebrae	2	1	2	2	1	1	2	2
- offset alignment pelvic girdle	—	—	2	1	—	—	2	1
Incomplete ossification/unossified								
- cranial centres	35	45	21	11*	14	16	10	7
- hyoid	—	1	2	1	—	1	2	1
- vertebrae:								
cervical	2	2	—	—	2	2	—	—
thoracic	—	3	—	1	—	3	—	1
lumbar	—	—	—	1	—	—	—	1
caudal	—	1	—	—	—	1	—	—
sacrocaudal	2	1	2	—	2	1	2	—
- sternebrae:								
5th and/or 6th	34	29	19	20	17	14	12	13
other	2	4	2	4	2	4	2	4
total	34	30	19	21	17	14	12	13
- pelvic bones	—	1	—	—	—	1	—	—
- metacarpals/metatarsals	—	2	1	—	—	2	1	—
Precocious ossification								
- cervical vertebral centra (> 5 ossified)	33	21	19	17	14	10	10	8
Additional observations at necropsy								
- left umbilical artery	11	8	4	7	8	5	4	7
- shiny skin	—	1	—	—	—	1	—	—

From Pattern (2006a)
* $P < 0.05$

[a] Fetuses with major abnormalities excluded; postscript letters represent one fetus with > 1 abnormality or variant. The study report stated: "Each postscript letter in the body of the table represents one fetus within a group with more than one abnormality/variant." "Each letter also indicates the abnormalities/variants occurring in the fetus." Postscripts: a = same fetus had: sternebrae with offset alignment, bipartite ossified/misshapen, costal cartilage had offset alignment, additional, partially fused; b = same fetus had: sternebrae partially fused, additional and costal cartilage had 2nd cartilage not connected to sternum.

Table 59. Group incidences of minor visceral abnormalities in rats

Site	Abnormality	Fetal incidences[a]				Litter incidences[a]			
		Dose (mg/kg bw per day)							
		0	62.5	250	1000	0	62.5	250	1000
Number examined		*127*	*124*	*115*	*108*	*22*	*22*	*22*	*21*
Number affected		*16*	*30**	*21**	*30**	*8*	*18*	*14*	*15*
Eye(s)	Variation in size	2	—	—	3	2	—	—	3
Thyroid	Rudimentary	—	—	—	1	—	—	—	1
Thymus	Partially undescended	—	1	2	3	—	1	2	3

Table 59 (continued)

Site	Abnormality	Fetal incidences[a]				Litter incidences[a]			
		Dose (mg/kg bw per day)							
		0	62.5	250	1000	0	62.5	250	1000
Innominate artery	Short	—	—	1	—	—	—	1	—
Azygos vein	Right-sided	1	—	—	—	1	—	—	—
Inferior vena cava	Anomalous confluence with left hepatic vein	—	—	—	1	—	—	—	1
Diaphragm	Thinning with protruding liver	4	4	7	7	2	4	6	7
Liver	Fissure/bilobed posterior caudate lobe	—	1	1	—	—	1	1	—
Kidney(s)	Dilated renal pelvis	—	—	1	—	—	—	1	—
Ureter(s)	Dilated	—	—	1	—	—	—	1	—
Testis(es)	Displaced	—	5	2	4[#]	—	4	2	4
Umbilical artery	Left-sided	9	15	7	11	8	11	5	8
Haemorrhages	Brain/spinal cord	2	—	1	—	2	—	1	—
	Intra-abdominal	—	1	—	3	—	1	—	3
	Hepatic	1	—	1	2	1	—	1	2
	Subcutaneous	—	4	—	1	—	3	—	1

From Pattern (2006a). Data taken from report table 11, p. 40.
[#] $P < 0.05$ (exact Wilcoxon rank sum test); * $P < 0.05$ (Williams' test)
[a] Individual fetuses/litters may occur in more than one category. Fetuses with major abnormalities excluded.

Table 60. Incidences of visceral abnormalities compared with historical control data in rats

Finding	Fetal (litter) incidence (%)			
	Dose (mg/kg bw per day)			
	0	62.5	250	1000
Partially undescended thymus	0.0 (—)	0.8 (4.5)	1.7 (9.1)	2.8 (14.3)
Thin diaphragm with protruding liver	3.1 (9.1)	3.2 (18.1)	6.1 (27.3)	6.5 (33.3)
Displaced testis(es)	0.0 (—)	4.0 (18.2)	1.7 (9.1)	3.7* (19.0)
Historical control ranges				
- partially undescended thymus	0.0–2.3% (= 2/88 fetuses in 2/19 litters)			
- thin diaphragm with protruding liver	0.0–8.0% (= 10/125 fetuses in 8/22 litters)			
- displaced testis(es)	0.0–5.5% (= 7/127 fetuses in 6/21 litters)			

From Pattern (2006a)
* $P < 0.05$

Rabbits

In a preliminary study of prenatal developmental toxicity, groups of six mated New Zealand White female rabbits were given penthiopyrad (purity 98.6%) suspended in a solution of 0.5% carboxymethylcellulose/0.1% Tween 80 by oral gavage at a dose of 0, 250, 500 or 1000 mg/kg bw per day from GD 6 to GD 28. All dams were sacrificed on GD 29, and the uterine contents were examined.

There was evidence of maternal toxicity at all doses tested, with body weight loss, decreased body weight gain and decreased feed consumption starting at 250 mg/kg bw per day and mortality at

Table 61. Group mean rabbit litter data

Dose (mg/kg bw per day)	CL	Implantations	Resorptions			Live young			Sex ratio	Implantation loss (%)	
			Early	Late	Total	Male	Female	Total	(% M)	Pre-	Post-
0	12.3	11.0	0.9	0.3	1.2	5.2	4.6	9.8	50.2	12.5	9.9
25	11.2	9.5	0.5	0.4	0.9	4.4	4.2	8.6	51.6	15.8	9.3
75	12.3	10.2	0.5	0.3	0.8	5.3	4.1	9.4	56.1	17.7	6.6
225	12.4	10.3	0.9	0.1	1.0	4.1	5.2	9.3	47.5	17.1	8.9

From Pattern (2006c)
CL, corpora lutea; M, male

500 mg/kg bw per day and above. Gravid uterine weight was also decreased at all doses. Increased pre-implantation loss and decreased mean litter weight were observed at all doses, with fetal weight also decreased at the highest dose tested.

The LOAEL for maternal and developmental toxicity was 250 mg/kg bw per day, based on body weight loss, decreased body weight gain and feed consumption, increased pre-implantation loss and decreased litter weight (Pattern, 2006b).

In a developmental toxicity study in rabbits, penthiopyrad (purity 98.6%) was administered to mated female New Zealand White rabbits (24 per dose) via gavage, from day 6 to day 28 of gestation, at a dose level of 0, 25, 75 or 225 mg/kg bw per day at 5 ml/kg bw in aqueous carboxymethylcellulose (0.5% w/v)/Tween 80 (0.1% 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 examination and skeletal examination.

There was no conclusive evidence of maternal effects of treatment. However, one female receiving 225 mg/kg bw per day was killed prematurely because of evidence of abortion on day 26 of gestation, which followed a period of markedly reduced feed consumption and weight loss. Based on its occurrence at the highest dose level, 225 mg/kg bw per day, the closeness of this dose level to the maximum tolerated dose and a low incidence of spontaneous abortion in this strain of rabbit, it was considered to be treatment induced. There were no other maternal clinical signs that could be related to treatment with penthiopyrad. Maternal body weight and feed consumption were unaffected by treatment at all dose levels. On day 29 of gestation, there were no treatment-related macroscopic observations at necropsy at any dose level. There were 23, 24, 22 and 21 live litters in the controls and 25, 75 and 225 mg/kg bw per day groups, respectively. Litter data, as assessed by the number of corpora lutea, implantations, resorptions and live young, and the extent of pre-implantation and post-implantation loss showed no adverse effects of treatment at any dose level (Table 61). At 225 mg/kg bw per day, the overall mean fetal weight was 7.8% lower than the control value. This was mainly attributable to a statistically significant 12.1% reduction in female mean fetal weight, which was also outside the historical control range (Table 62), although male fetal weight was also marginally lower than that of the controls. There was no associated evidence of retarded fetal development identified during detailed visceral and skeletal examinations. Mean litter weight at 225 mg/kg bw per day was also reduced by 13.5% relative to the controls. Fetal and litter weights at 25 or 75 mg/kg bw per day and placental weight at all dose levels were unaffected by maternal treatment with penthiopyrad (Table 63). The nature and incidences of major and minor fetal abnormalities and skeletal variants were unaffected by treatment with penthiopyrad at all dose levels employed.

Table 62. Historical control data in rabbits (seven studies)

	Placental weight (g)	Litter weight (g)	Male fetal weight (g)	Female fetal weight (g)	Overall fetal weight (g)
Mean	5.38	361.9	40.74	39.94	40.47
SD	0.19	28.6	0.78	0.71	0.86
Minimum	5.10	306.6	39.90	39.37	39.80
Maximum	5.60	389.4	42.30	41.40	42.30

From Pattern (2006c)
SD, standard deviation

Table 63. Group mean placental, fetal and litter weights in rabbits

Dose (mg/kg bw per day)	Placental weight (g) ± SD	Litter weight (g) ± SD	Fetal weight (g) ± SD		
			Males	Females	Overall
0	5.35 ± 1.03	376.4 ± 106.10	38.83 ± 5.42	39.78 ± 7.49	39.71 ± 6.83
25	5.26 ± 0.66	349.7 ± 91.70	41.96 ± 5.99	40.73 ± 5.73	41.41 ± 5.64
75	5.16 ± 0.83	358.0 ± 87.13	38.98 ± 6.23	38.58 ± 7.33	38.79 ± 6.44
225	5.15 ± 0.78	325.6 ± 86.40	37.45 ± 6.12	34.97 ± 5.16*	36.63 ± 6.43

From Pattern (2006c)
SD, standard deviation; * $P < 0.05$

The maternal NOAEL was 75 mg/kg bw per day, based on the occurrence of abortion in one animal at 225 mg/kg bw per day. The fetal NOAEL was also 75 mg/kg bw per day, based on the occurrence of slightly reduced fetal weight at 225 mg/kg bw per day (Pattern, 2006c).

2.6 Special studies

(a) Effects on hepatic drug-metabolizing enzyme induction and cell proliferation

The objective of a study by Harada (2009) was to investigate the effects of penthiopyrad on hepatic drug-metabolizing enzyme induction and cell proliferation in male mice 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.

Groups of 18 young male CD-1 strain mice were treated orally, by admixture in the diet, for up to 14 days with penthiopyrad (purity 98.6%; lot No. 2000111) at a target dose level of 0, 25, 60, 200 or 600 mg/kg bw per day. A similar group of mice was treated with phenobarbital at 1000 ppm for a similar period. Six animals per group were killed after 3, 7 or 14 days of treatment. The mean achieved dose levels of penthiopyrad, based on an average of all animals, were 0, 25.1, 61.6, 197 and 561 mg/kg bw per day.

All animals were killed and subjected to necropsy. Sections of liver were processed for the assessment of cell proliferation by 5-bromo-2′-deoxyuridine (BrdU) incorporation, apoptosis by terminal deoxynucleotidyl transferase deoxyuridine triphosphate (dUTP) nick end labelling (TUNEL) and histopathology. The hepatic microsomal fraction was subjected to analysis of protein content, cytochrome P450 (CYP) content and ethoxycoumarin *O*-dealkylase (ECOD) and pentoxyresorufin *O*-dealkylase (PROD) activities. In addition, protein contents of P450 isozymes (CYP1A, CYP2B, CYP3A and CYP4A) were determined by western blot analysis.

Table 64. Summary of liver weight data in mice

Dose (mg/kg bw per day)	Duration of treatment (days)	Group mean liver weight (% of control value)	
		Absolute	Relative
25	3	103	107
	7	104	106
	14	112	106
60	3	97	99
	7	101	103
	14	117*	111
200	3	103	106
	7	103	106
	14	110	109
600	3	113	114**
	7	113*	113**
	14	121**	120**
Phenobarbital (1000 ppm)	3	128**	131**
	7	155**	152**
	14	159**	149**

From Harada (2009)
* $P < 0.05$; ** $P < 0.01$

There were no deaths or treatment-related clinical signs at any dose level. There were no effects of treatment at any dose level of penthiopyrad on body weight gain or feed consumption. However, animals treated with phenobarbital showed 4–7% higher body weights than the controls. Gross necropsy findings were confined to dark-coloured liver in four of six animals treated with penthiopyrad at 600 mg/kg bw per day for 14 days and enlarged liver in a majority of animals treated with phenobarbital for 7 or 14 days. Group mean absolute and relative liver weights were increased after 3, 7 or 14 days of treatment with phenobarbital and 600 mg/kg bw per day penthiopyrad, but not at lower dose levels (Table 64).

Administration of phenobarbital and penthiopyrad at 600 mg/kg bw per day each produced a pattern of effects comprising increases in liver weight, microsomal P450 content, microsomal ECOD and PROD activities (Table 65), hepatic CYP1A, CYP2B and CYP3A isozyme activities (Table 66) and hepatic BrdU labelling index (Table 67), together with histological evidence of centrilobular hepatic hypertrophy. Microsomal P450 content and enzyme activities and hepatic P450 isozyme activities were also increased in response to penthiopyrad administration at 200 mg/kg bw per day. Phenobarbital also induced an increase in microsomal protein content. The effects of penthiopyrad on hepatic microsomal enzyme induction and cell proliferation have a threshold below which they do not occur, and the threshold value is considered to be 60 mg/kg bw per day.

Based on the similarity in response between phenobarbital and penthiopyrad, specifically the cell proliferation and hepatic enzyme induction responses, it was postulated by the study author that penthiopyrad is a hepatic enzyme inducer with the potential to enhance cell proliferation in the liver during the initial phase of treatment. It has been proposed that non-genotoxic mitogenic hepatocarcinogens such as phenobarbital may enhance hepatic cell proliferation, but the enhanced proliferation ceases after a few days, even if treatment is continued. Furthermore, non-genotoxic hepatocarcinogens with mitogenic activity may provide a selective growth advantage to spontaneously initiated preneoplastic cells over normal hepatocytes, leading to neoplasia (Harada, 2009).

Table 65. Summary of hepatic microsomal protein and P450 content and enzyme activity after 14 days in mice

Dose (mg/kg bw per day)	% of control value			
	Microsomal protein	P450	Microsomal enzyme activity	
			ECOD	PROD
25	107	104	107	180
60	112	119	119	580
200	118	143**	174**	2100**
600	118	191**	289**	4120**
Phenobarbital (1000 ppm)	128**	294**	486**	6800**

From Harada (2009)
** $P < 0.01$

Table 66. Summary of hepatic microsomal P450 isozyme content after 14 days in mice

Dose (mg/kg bw per day)	P450 isozyme content (% of control value)			
	CYP1A	CYP2B	CYP3A	CYP4A
25	134	273	91	46
60	134	283	124	67
200	204*	472**	198*	23
600	452**	995**	318**	28
Phenobarbital (1000 ppm)	325**	1799**	319**	23

From Harada (2009)
* $P < 0.05$; ** $P < 0.01$

Table 67. Summary of hepatocyte 5-bromo-2'-deoxyuridine labelling index after 3, 7 or 14 days in mice

Dose (mg/kg bw per day)	BrdU labelling index (% of control value)		
	3 days	7 days	14 days
25	120	83	157
60	160	92	186*
200	120	108	129
600	220	142	171
Phenobarbital (1000 ppm)	680**	242**	200

From Harada (2009)
* $P < 0.05$; ** $P < 0.01$

The objective of a study by Harada (2002, 2006) was to investigate the effects of penthiopyrad on hepatic drug-metabolizing enzyme induction and cell proliferation 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.

Groups of 18 young male Wistar rats were treated orally, by admixture in the diet for up to 14 days, with penthiopyrad (purity 99.8%) at a concentration of 0, 100, 1000 or 10 000 ppm (equal to

0, 6.47, 66.7 and 632 mg/kg bw per day, respectively) or 1000 ppm phenobarbitone or 3000 ppm clofibrate as reference materials. Six animals per group were sacrificed after 3, 7 and 14 days of treatment and subjected to necropsy, liver weight recording, measurement of hepatic cell proliferation by proliferating cell nuclear antigen (PCNA) immunohistochemical staining, analysis of gap junction protein (Cx32), as an indicator of cell-to-cell communication, in animals treated with penthiopyrad at 0 or 10 000 ppm and routine H&E liver histopathology. In addition, drug metabolizing enzyme activities in both the microsomal fraction and the 700 × *g* supernatant fraction containing peroxisomes were measured in animals sacrificed after 14 days:

- *Microsomal fraction*: protein content, cytochrome P450 content, PROD, uridine diphosphate glucuronosyltransferase (UDPGT) activity and CYP1A1, CYP2B1, CYP3A2 or CYP4A1 contents;
- *Hepatic 700 × g supernatant fraction containing peroxisomes*: protein content, palmitoyl-coenzyme A oxidase and carnitine acyltransferase activities.

Electron microscopy was performed on ultra-thin liver sections from two control and two high-dose penthiopyrad animals.

There were no deaths, adverse clinical signs or effects on feed consumption or body weight gain in any of the treatment groups. Animals treated with penthiopyrad at 10 000 ppm, two treated at 1000 ppm and phenobarbitone- or clofibrate-treated animals frequently showed dark-coloured and/or enlarged livers after 3, 7 and 14 days of treatment (Table 68). There were no other treatment-related gross lesions. Treatment with penthiopyrad at 10 000 ppm and with phenobarbitone or clofibrate elicited increased relative liver weight after 3, 7 and 14 days of treatment, but the effect was less marked with penthiopyrad than for the reference materials (Table 69). The hepatic 700 × *g* supernatant fraction containing peroxisomes was unaffected by all treatments except in response to clofibrate, for which protein content and palmitoyl-coenzyme A oxidase and carnitine acyltransferase activities were significantly increased (Table 70). In the microsomal fraction, PROD was increased approximately 6-fold at 10 000 ppm with penthiopyrad, but not at lower dose levels, and approximately 114-fold with phenobarbitone. UDPGT was also increased approximately 1.5-fold at 10 000 ppm penthiopyrad (Table 71). Cytochrome P450 isozymes were increased up to 6.45-fold at 10 000 ppm, and CYP4A1 was also increased at 1000 ppm, with increasing trends in the other isozymes. Phenobarbitone and clofibrate elicited up to 30-fold and 20-fold, respectively, increases in some P450 isozymes (Table 72). Cell proliferation showed an increasing trend at 10 000 ppm with penthiopyrad and with phenobarbitone treatment after 3 and 7 days of treatment. Cell proliferation was increased at 3 days only after clofibrate treatment (Table 73). There was no effect on cell-to-cell communication in the animals treated with 10 000 ppm penthiopyrad (Table 74). Animals treated with 10 000 ppm penthiopyrad and phenobarbitone-treated animals showed mild to moderate hypertrophy of centrilobular hepatocytes after 3, 7 and 14 days of treatment. Clofibrate-treated animals exhibited mild to moderate diffuse hepatocellular hypertrophy after 3, 7 or 14 days of treatment and increased cytoplasmic eosinophilia of hepatocytes after 14 days of treatment (Table 75). Electron microscopic evaluation of liver from two animals treated with penthiopyrad at 10 000 ppm revealed proliferation of smooth endoplasmic reticulum.

Penthiopyrad exhibited similar characteristics to a phenobarbitone-type enzyme inducer. The NOAEL for hepatic enzyme induction was established at 100 ppm penthiopyrad (equal to a dose level of 6.47 mg/kg bw per day) (Harada, 2002, 2006).

The objective of a study by Harada (2008) was to investigate the effects of penthiopyrad on hepatic drug-metabolizing enzyme induction and cell proliferation 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.

Table 68. Incidence of macroscopic lesions in liver of rats

Dietary concentration (ppm)	Incidence					
	Enlarged liver			Dark-coloured liver		
	3 days	7 days	14 days	3 days	7 days	14 days
0	0/6	0/6	0/6	0/6	0/6	0/6
100	0/6	0/6	0/6	0/6	0/6	0/6
1000	0/6	2/6	0/6	0/6	0/6	0/6
10 000	2/6	6/6**	2/6	4/6*	0/6	3/6
Phenobarbitone (1000)	2/6	5/6**	6/6**	3/6	1/6	4/6*
Clofibrate (3000)	4/6*	6/6**	6/6**	2/6	1/6	4/6*

From Harada (2002, 2006)
* $P < 0.05$; ** $P < 0.01$ (Fisher's exact test)

Table 69. Group mean absolute and relative liver weights in rats

Dietary concentration (ppm)	Group mean absolute liver weight (g) ± SD			Group mean relative liver weight (%) ± SD		
	3 days	7 days	14 days	3 days	7 days	14 days
0	10.81 ± 0.84	11.11 ± 0.98	10.66 ± 1.59	3.67 ± 0.16	3.57 ± 0.23	3.28 ± 0.35
100	11.00 ± 0.81	11.55 ± 1.28	10.91 ± 0.90	3.76 ± 0.17	3.66 ± 0.18	3.37 ± 0.18
1000	11.66 ± 0.52	11.47 ± 1.51	11.56 ± 0.61	3.95 ± 0.17	3.69 ± 0.37	3.55 ± 0.16
10 000	12.10 ± 1.69	12.43 ± 0.32	12.37 ± 1.24	4.16 ± 0.39**	4.01 ± 0.17*	3.79 ± 0.16**
Phenobarbitone (1000)	12.92 ± 1.32§§	14.61 ± 1.89§§	14.30 ± 1.10§§	4.31 ± 0.21§§	4.56 ± 0.28§§	4.37 ± 0.25§§
Clofibrate (3000)	13.87 ± 1.84§§	15.17 ± 2.15§§	15.99 ± 3.10§§	4.76 ± 0.54§§	4.86 ± 0.42§§	4.99 ± 0.70§§

From Harada (2002, 2006)
SD, standard deviation; * $P < 0.05$; ** $P < 0.01$ (Dunnett's test); §§ $P < 0.01$ (Student's *t*-test)

Table 70. Group mean enzyme activities in the rat hepatic 700 × g supernatant containing peroxisomes

Dietary concentration (ppm)	Protein content (700 × *g* supernatant) (mg/g liver)	PCO (nmol/min per milligram protein)	CAT (nmol/h per milligram protein)
0	159 ± 17	3 ± 2	15 ± 6
100	156 ± 13	4 ± 1	19 ± 6
1000	154 ± 16	4 ± 4	15 ± 3
10 000	147 ± 12	4 ± 2	16 ± 5
Phenobarbitone (1000)	165 ± 10	3 ± 2	12 ± 3
Clofibrate (3000)	187 ± 7§§	98 ± 25§§	209 ± 38§§

From Harada (2002, 2006)
CAT, carnitine acyltransferase; PCO, palmitoyl-coenzyme A oxidase; §§ $P < 0.01$ (Student's *t*-test or Aspin-Welch test)

Table 71. Group mean rat hepatic microsomal enzyme activities

Dietary concentration (ppm)	Microsomal protein (mg/g liver) ± SD	Cytochrome P450 (nmol/mg protein) ± SD	PROD (pmol/min per milligram protein) ± SD	UDPGT (nmol/h per milligram protein) ± SD
0	46 ± 7	0.51 ± 0.07	12 ± 5	0.027 ± 0.008
100	44 ± 2	0.45 ± 0.04	12 ± 4	0.027 ± 0.003

Table 71 (continued)

Dietary concentration (ppm)	Microsomal protein (mg/g liver) ± SD	Cytochrome P450 (nmol/mg protein) ± SD	PROD (pmol/min per milligram protein) ± SD	UDPGT (nmol/h per milligram protein) ± SD
1000	44 ± 2	0.45 ± 0.07	14 ± 7	0.034 ± 0.006
10 000	51 ± 3	0.53 ± 0.0	72 ± 17**	0.040 ± 0.005**
Phenobarbitone (1000)	52 ± 5	1.19 ± 0.09§§	1370 ± 163§§	0.029 ± 0.003
Clofibrate (3000)	57 ± 5§§	0.82 ± 0.07§§	20 ± 14	—

From Harada (2002, 2006)
— not examined; PROD, pentoxyresorufin *O*-dealkylase; SD, standard deviation; UDPGT, uridine diphosphate glucuronosyltransferase; ** $P < 0.01$ (Dunnett's multiple comparison test); §§ $P < 0.01$ (Student's *t*-test or Aspin-Welch test)

Table 72. Group mean rat hepatic microsomal cytochrome P450 isozyme content

Dietary concentration (ppm)	Cytochrome P450 isozyme content (pmol/mg protein) ± standard deviation			
	CYP1A1	CYP2B1	CYP3A2	CYP4A1
0	0.000	8.06 ± 1.07	86.8 ± 13.8	7.82 ± 1.59
100	0.000	7.82 ± 1.24	80.5 ± 14.9	9.53 ± 3.84
1000	0.000	11.6 ± 2.2	116 ± 23	18.0 ± 6.5**
10 000	0.000	52.0 ± 10.8**	139 ± 30**	16.9 ± 5.6**
Phenobarbitone (1000)	0.000	246 ± 22§§	207 ± 38§§	13.3 ± 3.6§
Clofibrate (3000)	0.509 ± 0.036##	14.4 ± 0.7§§	119 ± 5§§	154 ± 40§§

From Harada (2002, 2006)
** $P < 0.01$ (Dunnett's multiple comparison test); § $P < 0.05$ (Student's *t*-test or Aspin-Welch test); §§ $P < 0.01$ (Student's *t*-test or Aspin-Welch test); ## $P < 0.01$ (Mann-Whitney U test)

Table 73. Group mean PCNA labelling index in rat hepatocytes

Dietary concentration (ppm)	Mean PCNA labelling index ± standard deviation		
	3 days	7 days	14 days
0	0.35 ± 0.31	0.38 ± 0.32	0.18 ± 0.13
100	0.38 ± 0.29	0.42 ± 0.49	0.18 ± 0.07
1000	0.37 ± 0.18	0.43 ± 0.34	0.28 ± 0.13
10 000	0.66 ± 0.91	1.01 ± 0.33*	0.28 ± 0.03
Phenobarbitone (1000)	3.39 ± 1.25§§	1.15 ± 0.64§	0.31 ± 0.11
Clofibrate (3000)	2.06 ± 1.53§	0.71 ± 0.30	0.28 ± 0.27

From Harada (2002, 2006)
* $P < 0.05$ (Dunnett's multiple comparison test); § $P < 0.05$; §§ $P < 0.01$ (Student's *t*-test or Aspin-Welch test)

Table 74. Mean number of Cx32 spots in the rat liver

Dietary concentration (ppm)	Mean number of Cx32 spots ± standard deviation		
	3 days	7 days	14 days
0	5.5 ± 0.2	5.5 ± 0.2	5.6 ± 0.2
10 000	5.5 ± 0.3	5.4 ± 0.1	5.5 ± 0.2

From Harada (2002, 2006)

Table 75. Nature and incidence of microscopic lesions in rats

Dietary concentration (ppm)	Incidence								
	Centrilobular hepatocellular hypertrophy			Diffuse hepatocellular hypertrophy			Cytoplasmic eosinophilia of hepatocytes		
	3 days	7 days	14 days	3 days	7 days	14 days	3 days	7 days	14 days
0	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
100	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
1000	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
10 000	6/6**	6/6**	6/6**	0/6	0/6	0/6	0/6	0/6	0/6
Phenobarbitone (1000)	6/6**	6/6**	6/6**	0/6	0/6	0/6	0/6	0/6	0/6
Clofibrate (3000)	0/6	0/6	0/6	6/6**	6/6**	6/6**	0/6	0/6	6/6**

From Harada (2002, 2006)
** $P < 0.01$ (Fisher's exact test)

Two groups of six young male Wistar rats were treated orally, by admixture in the diet, for 7 or 14 days with 0, 400, 4000 or 16 000 ppm penthiopyrad (purity 98.6%; lot No. 2000111) and then sacrificed and subjected to necropsy. A further group of six animals similarly treated for 14 days was sacrificed after a 28-day recovery period. The mean achieved dose levels, based on an average of all animals including the recovery group, were 0, 37.8, 371 and 1453 mg/kg bw per day. Circulating serum levels of thyroxine (T_4), triiodothyronine (T_3) and thyroid stimulating hormone (TSH) were measured at the end of the treatment or recovery periods, and hepatic microsomal UDPGT activities, protein content and total cytochrome P450 activity were measured. Quantitative analysis of gene expression related to TSH in the pituitary was performed, and thyroid follicular cell proliferation activity was measured. Pituitary, thyroid and liver tissues were stained with H&E and examined microscopically. In addition, further paraffin sections of the pituitary from each animal were stained immunohistochemically for TSH-secreting cells.

There were no deaths or adverse clinical signs in any of the treatment groups. Effects on body weight were confined to the group treated at 16 000 ppm, which had group mean body weights 12–15% lower than control values at termination. Effects on body weight were reversible. Feed consumption at 16 000 ppm was markedly reduced during the 1st week of treatment, but the effect was not evident at lower dose levels.

Penthiopyrad at dietary concentrations of 4000 and/or 16 000 ppm elicited a pattern of effects comprising hepatocellular hypertrophy, increased liver weight, increased hepatic cytochrome P450 and UDPGT activities, reduced circulating T_4 activity, upregulation of the pituitary *Prop-1* gene, increased TSH activity, increased thyroid follicular cell proliferation and thyroid follicular cell hypertrophy (Tables 76–80). The effects of treatment were fully reversible on withdrawal of treatment for 28 days. Based on these results, it is postulated that penthiopyrad is a phenobarbital-type hepatic UDPGT inducer with a potential to enhance biliary excretion of T_4, thereby lowering circulating T_4 levels, which results in an increase in circulating TSH through negative feedback, leading to thyroid follicular cell hypertrophy. Furthermore, prolonged follicular cell hypertrophy under the influence of increased TSH is considered to be a rational basis for the development of a slightly increased number of thyroid adenomas in the 104-week study (Blumbach, 2006b). In addition, it is concluded that the effects of penthiopyrad on thyroid function, and thus on neoplasia in the long-term study, have a threshold below which thyroid neoplasia will not occur.

Table 76. Group mean circulating T_3, T_4 and TSH activities in rats

Dietary concentration (ppm)	% of control value								
	T_3			T_4			TSH		
	7 days	14 days	28-day recovery	7 days	14 days	28-day recovery	7 days	14 days	28-day recovery
400	98	87	94	102	107	83	130*	77	115
4000	98	89	97	102	96	101	129	113	123
16 000	83	88	104	49**	58**	104	138*	113	248

From Harada (2008)
T_3, triiodothyronine; T_4, thyroxine; TSH, thyroid stimulating hormone; * $P < 0.05$; ** $P < 0.01$

Table 77. Group mean organ weights in rats

Dietary concentration (ppm)	Day	% of controls			
		Thyroid weight		Liver weight	
		Absolute	Relative	Absolute	Relative
400	7	110	109	103	102
	14	89	88	101	100
	28-day recovery	109	100	111	102
4000	7	111	111	117	116**
	14	91	90	114	113**
	28-day recovery	113	108	103	99
16 000	7	98	111	121*	137**
	14	95	111	119**	140**
	28-day recovery	152	151	107	109

From Harada (2008)
* $P < 0.05$; ** $P < 0.01$

Table 78. Group mean rat hepatic microsomal analyses

Dietary concentration (ppm)	Day	% of controls			
		Microsomal protein	Cytochrome P450	UDPGT activity towards:	
				4-nitrophenol	4-hydroxybiphenyl
400	7	119**	108	109	110
	14	108	97	118	127
	28-day recovery	92	106	94	96
4000	7	126**	144**	135	253*
	14	104	123**	118	240*
	28-day recovery	92	106	89	96
16 000	7	140**	156**	322**	410**
	14	102	135**	418**	410**
	28-day recovery	94	97	89	84

From Harada (2008)
* $P < 0.05$; ** $P < 0.01$

Table 79. Group mean rat thyroid follicular cell PCNA labelling index

Dietary concentration (ppm)	PCNA labelling index (% of control)		
	7 days	14 days	After 28 days' recovery
400	167	105	114
4000	206**	95	86
16 000	200	118	129

From Harada (2008)
PCNA, proliferating cell nuclear antigen; ** $P < 0.01$

Table 80. Group mean gene expression in the rat pituitary

Dietary concentration (ppm)	Day	% of control	
		Prop-1	
		Concentration	Ratio to GAPDH
400	7	104	117
	14	116	132
	28-day recovery	108	108
4000	7	138	143
	14	112	113
	28-day recovery	128	120
16 000	7	149*	157
	14	142	165
	28-day recovery	109	105

From Harada (2008)
GADPH, glyceraldehyde-3-phosphate dehydrogenase; * $P < 0.05$

The NOAEL for the postulated mechanism is 400 ppm (equal to 37.8 mg/kg bw per day) (Harada, 2008).

(b) Neurotoxicity

In an acute neurotoxicity study, penthiopyrad (purity 98.6%) was administered to young adult Sprague-Dawley strain rats (10 of each sex per dose) via gavage in 10 ml/kg bw of an aqueous suspension 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 FOB of tests, including a quantitative assessment of motor activity, was performed on all animals pre-dosing, on the day of treatment, at the time of peak effect (Chapman, 2008a) and on days 8 and 15. The time to peak effect was determined as 4 hours post-treatment in a preliminary study. All animals 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. Six animals of each sex from the control and high-dose groups were subjected to histopathological evaluation.

No premature deaths occurred, and there were no adverse clinical signs at the routine observation intervals. Body weight gain of both sexes at 2000 mg/kg bw was reduced by approximately 9% in males and 10% in females during the 1st week of the observation period (Table 81).

In the FOB, home cage observations were unaffected by treatment at all dose levels on all testing occasions. During the in-the-hand (Table 82), arena (Table 83), manipulation (Table 84) and

Table 81. Group mean body weight and overall weight gain in rats

Dose (mg/kg bw)	Mean body weight (g ± SD) on day:			Gain (g ± SD)
	1	8	15	Days 1–15
Males				
0	230 ± 15.6	307 ± 23.3	349 ± 27.3	119 ± 18.2
125	231 ± 17.3	304 ± 23.2	346 ± 30.3	115 ± 15.4
500	231 ± 17.3	308 ± 13.7	344 ± 20.3	113 ± 16.0
2000	224 ± 14.5	293 ± 19.7	332 ± 28.6	109 ± 19.0
Females				
0	184 ± 186	226 ± 21.8	237 ± 24.1	53 ± 6.8
125	177 ± 10.5	216 ± 18.4	228 ± 22.0	51 ± 12.8
500	183 ± 9.0	224 ± 14.6	240 ± 17.9	57 ± 9.8
2000	174 ± 7.6	211 ± 15.2	225 ± 16.4	51 ± 10.0

From Chapman (2008b)
SD, standard deviation

Table 82. Incidence of in-the-hand observations in rats: day 1 (time of peak effect)

Parameter	Incidence (no. of animals)							
	Males				Females			
	Dose (mg/kg bw)							
	0	125	500	2000	0	125	500	2000
No. of animals examined	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*
Ease of removal								
- some resistance or avoidance	2	1	1	0	1	0	2	0
- difficult; considerable avoidance/resistance	0	0	0	0	1	1	0	0
Piloerection (present)	0	0	1	6	0	1	1	5
Fur condition (slightly matted)	2	1	0	0	0	4	0	1
Vocalization (vocalizing softly)	0	0	0	1	0	0	0	1
Reactivity to handling								
- easy: awareness but little resistance	5	6	5	8	4	7	10	10
- slightly awkward: some struggling	5	4	5	2	6	3	0	0
Brown coat staining	2	1	0	0	0	2	0	1
Hairloss: forelimbs	0	0	0	0	0	2	0	0
Reduced body tone	0	0	0	7	0	0	1	6
Chewing mouth movements	0	0	0	1	0	0	0	0

From Chapman (2008b)

motor activity assessments (Table 85) on the day of dosing, the following treatment-related effects occurred in one or both sexes treated at 500 or 2000 mg/kg bw: reduced reactivity to handling, reduced body tone, abnormal gait, hunched posture, reduced body temperature, increased landing foot splay, no response in the approach and touch tests, and reduced motor activity. In addition, piloerection, whole body tremor, chewing mouth movements, slow breathing and weak response in the tail-pinch test occurred at 2000 mg/kg bw only. A slight decrease in the body temperature of one female at 125 mg/kg bw and marginally lower motor activity in both sexes at 125 mg/kg bw were

Table 83. Incidence of arena observations in rats: day 1 (time of peak effect)

Parameter (grade range)	Incidence (no. of animals)							
	Males				Females			
	Dose (mg/kg bw)							
	0	125	500	2000	0	125	500	2000
No. of animals examined	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*
Palpebral closure								
- eyelids slightly drooping	2	0	1	2	0	1	0	0
- eyelids half-closed	2	5	5	4	0	0	0	2
Posture: hunched	0	0	2	5	0	0	1	5
Gait								
- unable to assess[a]	4	5	6	6	0	0	0	1
- slightly abnormal	0	0	0	2	0	1	8	8
- moderately abnormal	0	0	0	1	0	0	0	1
Arousal: reduced awareness	2	5	6	5	0	0	0	0
Tremor								
- slight	1	3	5	3	0	0	2	7
- occasional	1	1	4	3	0	0	2	7
- frequent	0	2	1	0	0	0	0	0
Activity count (mean ± SD)	8.1 ± 6.2	9.6 ± 8.1	5.0 ± 4.7	6.3 ± 7.0	20.7 ± 7.3	24.1 ± 8.5	23.6 ± 6.1	16.5 ± 8.9
Rearing count (mean ± SD)	3.6 ± 3.1	4.6 ± 4.5	2.0 ± 2.4	2.6 ± 3.6	10.0 ± 5.7	11.1 ± 6.1	8.0 ± 2.9	6.1 ± 4.6
Grooming								
- no grooming	5	5	5	4	0	0	0	1
- occasional (< 4)	5	5	5	6	9	10	10	9
- numerous (> 4)	0	0	0	0	1	0	0	0
Urination amount								
- no urine	6	7	6	5	9	8	6	8
- small	1	2	1	3	0	0	1	0
- moderate	2	1	2	0	0	1	3	2
- large/excessive	1	0	1	2	1	1	0	0
Faecal count[b]								
- 0 pellets	7	8	6	6	10	10	10	10
- 1–2 pellets	2	0	1	3	0	0	0	0
- 3–4 pellets	1	2	3	1	0	0	0	0
Palpebral closure								
- single short period	0	0	0	1	0	0	0	0
- occasional short periods	1	0	3	1	0	1	0	0
Palpebral closure								
- single extended period	2	4	3	4	0	0	0	2
- occasional extended periods	1	1	0	0	0	0	0	0
Gait								
- slow	0	0	0	1	0	0	0	0
- elevated	0	0	0	1	0	1	1	5
- unsteady	0	0	0	2	0	0	8	8

Table 83 (continued)

Parameter (grade range)	Incidence (no. of animals)							
	Males				Females			
	Dose (mg/kg bw)							
	0	125	500	2000	0	125	500	2000
Licking surface of arena	1	1	0	0	0	1	2	0
Head shake	0	0	3	0	1	2	3	3
Head shake: frequent	0	0	0	0	0	1	1	0
Whole body shake: once/ occasional	0	0	0	1	0	2	2	1
Chewing mouth movements	1	0	3	3	1	1	3	5
Piloerection	0	0	1	1	0	0	1	1
Breathing: slow	0	1	0	3	0	0	0	0

From Chapman (2008b)
SD, standard deviation
[a] No or minimal locomotion during the observation period.
[b] Number of faecal pellets at the end of the testing period.

considered to be minimal differences and non-adverse. None of these effects was apparent at the subsequent evaluations on days 8 and 15.

Macroscopic examination at necropsy revealed no treatment-related lesions at any dose level. Brain weights and dimensions were unaffected by treatment at all dose levels. There were no treatment-related histopathological findings in any of the tissues of the central and peripheral nervous systems and skeletal muscle examined at 2000 mg/kg bw.

The NOAEL was 125 mg/kg bw, based on transient functional alterations (e.g. hunched posture, unsteady gait, reduced body temperature and increased landing foot splay) and decreased motor activity at the estimated time to peak effect (4 hours) on the day of administration observed at 500 mg/kg bw (Chapman, 2008b).

In a 13-week neurotoxicity study, penthiopyrad (purity 98.6%) was administered in the diet to young adult Sprague-Dawley rats (10 of each sex per dose) at a target dose level of 0, 10, 40, 160 or 640 mg/kg bw per day for 13 weeks. The overall mean achieved dose levels were 0, 11.0, 43.8, 177 and 712 mg/kg bw per day for males and 0, 10.7, 42.5, 170 and 686 mg/kg bw per day for females. Feed consumption and body weights were recorded weekly, and a FOB of tests, including a quantitative assessment of motor activity, was performed on all animals pre-dosing and during weeks 2, 4, 8 and 13. All animals 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. Six animals of each sex from the control and high-dose groups were subjected to histopathological evaluation.

A control female was sacrificed for humane reasons on day 12 after sustaining a fractured spinal column. No other premature deaths occurred, and there were no adverse clinical signs at the routine observation intervals. A treatment-related decrease in overall body weight gain of 11–17% occurred in both sexes at 640 mg/kg bw per day and in females at 160 mg/kg bw per day (Table 86). This effect was considered adverse only in males, as the effects in females did not demonstrate a dose–response

Table 84. Incidence of manipulation observations in rats: day 1 (time of peak effect)

Parameter (grade range)	Incidence (no. of animals)							
	Males				Females			
	Dose (mg/kg bw)							
	0	125	500	2000	0	125	500	2000
Number of animals	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*
Approach response								
- no reaction	0	1	5	9	1	2	3	4
- fearful or aggressive reaction	0	0	0	0	0	1	0	0
Touch response								
- no reaction	0	0	3	4	0	1	1	2
Auditory startle reflex: weak response	0	0	1	1	0	0	0	1
Tail pinch								
- no response	0	1	0	0	0	0	0	0
- weak response	0	1	1	4	0	0	2	1
Body temperature (°C) (mean ± SD)	37.0 ± 0.3	36.8 ± 0.3	36.6 ± 0.4*	36.3 ± 0.5**	37.2 ± 0.6	36.6 ± 0.5*	35.7 ± 0.7**	35.4 ± 0.6**
Body weight (g) (mean ± SD)	226 ± 16	228 ± 17	228 ± 10	221 ± 14	181 ± 19	174 ± 11	181 ± 9	173 ± 8
Landing foot splay (mm) (mean ± SD)	106 ± 16	107 ± 24	124 ± 19*	124 ± 14*	100 ± 22	95 ± 27	113 ± 22	114 ± 20
Forelimb grip strength (kg) (mean ± SD)	0.90 ± 0.08	0.90 ± 0.08	0.90 ± 0.12	0.86 ± 0.11	0.87 ± 0.11	0.87 ± 0.11	0.79 ± 0.10	0.84 ± 0.10
Hindlimb grip strength (kg) (mean ± SD)	0.35 ± 0.04	0.35 ± 0.05	0.36 ± 0.04	0.32 ± 0.05	0.32 ± 0.04	0.34 ± 0.04	0.32 ± 0.04	0.31 ± 0.03
Reduced body tone	0	0	0	0	0	0	1	1
Chewing mouth movements	1	0	0	0	0	1	0	0
Tremor during temperature recording	0	0	1	1	0	0	0	1
Tremor during handling								
- occasional	0	1	4	0	0	0	3	1
- frequent	0	1	1	0	0	0	0	2
Excessive vocalization during:								
- handling	0	0	0	1	0	0	1	0
- grip strength	1	0	0	0	0	0	0	0
Animal cold to touch	0	0	0	0	0	0	2	1

From Chapman (2008b)
SD, standard deviation; * $P < 0.05$; ** $P < 0.01$

Table 85. Group mean motor activity counts in rats: day 1 (time of peak effect)[a]

Dose (mg/kg bw)	Time (min) ± standard deviation										
	6	12	18	24	30	36	42	48	54	60	Total
Male											
High beam counts											
0	116.6 ± 18.0	49.5 ± 20.9	22.1 ± 18.4	4.0 ± 8.6	4.4 ± 10.7	0.0 ± 0.0	2.1 ± 5.7	3.2 ± 10.1	0.0 ± 0.0	3.2 ± 8.2	205.1 ± 33.6
125	89.9 ± 25.6*	40.8 ± 22.3	13.6 ± 24.4	0.0 ± 0.0	0.7 ± 2.2	0.3 ± 0.9	1.4 ± 4.4	9.7 ± 23.3	7.3 ± 15.4	1.2 ± 2.6	164.9 ± 82.0
500	80.2 ± 27.3**	22.0 ± 17.7**	0.3 ± 0.9**	0.0 ± 0.0	1.7 ± 5.4	1.1 ± 3.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	7.7 ± 22.6	113.0 ± 36.1**
2000	74.7 ± 32.2**	18.6 ± 14.5**	0.9 ± 2.5**	0.6 ± 1.9	0.1 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	5.9 ± 18.7	3.9 ± 12.3	0.1 ± 0.3	104.8 ± 36.9**
Low beam counts											
0	246.2 ± 51.4	145.1 ± 42.2	81.3 ± 45.2	18.7 ± 27.2	20.7 ± 31.1	1.6 ± 3.4	14.0 ± 32.1	16.1 ± 44.5	1.4 ± 2.8	25.2 ± 37.4	570.3 ± 154.8
125	261.7 ± 62.9	108.8 ± 41.4	52.3 ± 38.9	4.9 ± 5.4	7.5 ± 14.4*	3.5 ± 6.4	9.5 ± 24.9	44.8 ± 92.9	26.2 ± 54.6	9.0 ± 12.9	528.2 ± 220.0
500	235.0 ± 48.7	73.8 ± 51.0**	22.8 ± 31.9**	0.7 ± 1.9*	8.7 ± 24.4*	10.9 ± 27.8	0.3 ± 0.7	1.8 ± 4.7	1.0 ± 1.3	31.0 ± 77.6	386.0 ± 144.4*
2000	241.7 ± 70.9	75.8 ± 48.3**	3.1 ± 5.1**	5.1 ± 13.2*	0.7 ± 1.3**	0.2 ± 0.6	6.2 ± 13.3	10.9 ± 33.1	5.8 ± 14.7	6.1 ± 10.3	355.6 ± 71.5**
Female											
High beam counts											
0	110.3 ± 28.8	71.4 ± 43.2	63.4 ± 43.4	37.9 ± 32.8	14.8 ± 26.1	12.2 ± 24.2	18.7 ± 26.0	9.3 ± 11.0	13.0 ± 24.6	10.1 ± 22.8	361.1 ± 167.3
125	110.8 ± 25.1	60.8 ± 21.3	32.7 ± 24.4*	21.5 ± 24.8	17.6 ± 22.0	7.8 ± 13.5	7.7 ± 15.4	5.2 ± 11.1	4.6 ± 9.7	7.1 ± 12.9	275.8 ± 85.8
500	71.8 ± 25.9**	19.4 ± 20.3**	19.6 ± 20.6**	5.0 ± 9.7**	9.8 ± 21.3	1.1 ± 3.5	2.3 ± 7.3	9.2 ± 29.1	0.0 ± 0.0*	4.1 ± 10.9	142.3 ± 74.8**
2000	62.8 ± 30.1**	15.7 ± 31.7**	9.6 ± 18.2**	5.8 ± 10.1**	3.4 ± 10.8	0.6 ± 1.3	0.0 ± 0.0	3.9 ± 8.8	0.4 ± 1.3	1.9 ± 5.7	104.1 ± 96.5**
Low beam counts											
0	292.0 ± 23.8	160.9 ± 43.7	123.1 ± 53.4	87.6 ± 53.3	41.1 ± 38.1	29.8 ± 30.1	51.5 ± 55.9	49.0 ± 44.6	47.4 ± 60.6	48.1 ± 46.6	930.5 ± 229.4
125	245.9 ± 50.3*	134.6 ± 34.4	67.8 ± 46.6*	63.0 ± 53.0	63.0 ± 60.3	22.2 ± 24.8	15.6 ± 24.7	19.8 ± 38.5	19.0 ± 33.3	28.5 ± 31.5	679.4 ± 249.1*
500	233.8 ± 42.0**	87.9 ± 61.8**	61.9 ± 55.6*	22.4 ± 22.2**	24.3 ± 51.2	9.1 ± 17.7	13.9 ± 19.5	12.8 ± 35.3	6.5 ± 11.4*	26.0 ± 36.9	498.6 ± 163.4**
2000	196.8 ± 53.2**	82.0 ± 56.2**	56.6 ± 82.1*	23.9 ± 41.2**	10.8 ± 29.3	13.2 ± 27.0	5.4 ± 12.7**	16.3 ± 32.3	12.3 ± 24.8*	14.6 ± 42.3	431.9 ± 272.1**

From Chapman (2008b)

* $P < 0.05$; ** $P < 0.01$

[a] Historical control data (three studies):

High beam counts, male: Total activity counts: mean 230.7, minimum 215.5, maximum 248.3

Low beam counts, male: Total activity counts: mean 757.5, minimum 732.5, maximum 793.6

High beam counts, female: Total activity counts: mean 342.7, minimum 200.1, maximum 487.2

Low beam counts, female: Total activity counts: mean 836.5, minimum 639.2, maximum 1040.7

Table 86. Selected group mean body weights in rats

Dose (mg/kg bw per day)	Group mean body weight (g) ± SD						Body weight gain (g) ± SD
	Week						
	0	1	2	4	8	13	0–13
Males							
0	219 ± 14.7	273 ± 19.0	323 ± 21.1	386 ± 23.2	473 ± 33.6	544 ± 43.7	325 ± 38.3
10	224 ± 14.1	282 ± 13.7	329 ± 15.0	394 ± 23.4	484 ± 36.6	560 ± 40.9	336 ± 39.3
40	218 ± 15.4	271 ± 22.1	314 ± 29.0	379 ± 39.2	471 ± 50.8	543 ± 59.9	325 ± 49.0
160	222 ± 14.2	276 ± 19.4	318 ± 24.4	377 ± 30.2	460 ± 35.1	533 ± 43.6	311 ± 33.9 (↓5)[a]
640	228 ± 14.5	264 ± 13.8	301 ± 19.3	355 ± 25.7	430 ± 39.6	499 ± 51.0	271 ± 44.2** (↓17)
Females							
0	165 ± 14.2	189 ± 19.2	206 ± 21.8	229 ± 29.1	261 ± 36.5	286 ± 44.5	122 ± 34.4
10	175 ± 10.2	200 ± 13.6	215 ± 17.8	241 ± 16.9	271 ± 21.7	294 ± 19.1	119 ± 14.9
40	161 ± 15.5	184 ± 22.1	201 ± 27.8	225 ± 32.4	256 ± 38.3	282 ± 37.4	121 ± 24.8
160	173 ± 9.8	193 ± 13.7	209 ± 12.9	234 ± 16.4	262 ± 20.9	279 ± 24.1	106 ± 17.7 (↓13)
640	171 ± 11.1	191 ± 12.1	202 ± 15.6	228 ± 16.3	256 ± 18.6	279 ± 22.4	108 ± 17.1 (↓11)

From Groom (2008b)
SD, standard deviation; ** $P < 0.01$
[a] Per cent change compared with control.

relationship or result in a significant effect on body weight. Feed consumption for all treated groups was unaffected by treatment. Home cage, in-the-hand, arena and manipulation observations did not identify any treatment-related changes at any of the observation intervals during the treatment period (Table 87). Quantitative assessment of motor activity revealed no effects of treatment at any dose level at any observation interval on rearing activity or cage floor activity (Table 88). The macroscopic examination performed at termination revealed no lesions attributable to treatment with penthiopyrad at any dose level; brain weight, length and width were also unaffected by treatment at all dose levels. There were no treatment-related histopathological findings in any of the central and peripheral nerve tissues or in skeletal muscle, eyes or optic nerve in animals treated at 640 mg/kg bw per day.

The NOAEL for systemic effects was 160 mg/kg bw per day, based on the occurrence of reduced weight gain in males at 640 mg/kg bw per day. It was concluded that penthiopyrad did not elicit functional or morphological evidence of neurotoxicity at target dose levels up to the maximum tolerated dose of 640 mg/kg bw per day. Therefore, the NOAEL for neurotoxicity was 640 mg/kg bw per day (Groom, 2008b).

In a preliminary developmental neurotoxicity study, penthiopyrad (purity 99.2%) was administered to mated Crl:CD (SD) IGS BR female rats (eight per dose) from GD 6 to LD 7, and their pups were directly treated via gavage from day 7 to day 20/21 of age, at a dose of 0, 100, 300 or 1000 mg/kg bw per day. During the study, clinical condition, body weight, feed consumption, gestation length and parturition observations, macroscopic pathology and brain weight investigations were performed on the adult females. Clinical condition, litter size and offspring survival, sex ratio, body weight, macroscopic pathology and brain weight investigations were undertaken on the litters/offspring. Motor activity was also assessed on selected offspring on day 22.

Treatment of the maternal animals at doses up to 1000 mg/kg bw per day was well tolerated throughout the treatment period in the preliminary study, but pup weight loss, deterioration in clinical condition and offspring death occurred at 1000 mg/kg bw per day, and slight, transiently

Table 87. Selected group mean grip strength data in rats

	Week	Grip strength (kg) ± SD				
		Dose (mg/kg bw per day)				
		0	10	40	160	640
Males						
Forelimb	2	0.97 ± 0.13	0.92 ± 0.11	0.93 ± 0.11	0.97 ± 0.09	0.98 ± 0.09
Hindlimb		0.72 ± 0.08	0.66 ± 0.07	0.68 ± 0.08	0.70 ± 0.06	0.70 ± 0.10
Forelimb	4	1.09 ± 0.13	1.08 ± 0.11	1.00 ± 0.13	1.02 ± 0.20	1.08 ± 0.14
Hindlimb		0.84 ± 0.15	0.80 ± 0.14	0.80 ± 0.13	0.76 ± 0.14	0.83 ± 0.13
Forelimb	8	1.27 ± 0.23	1.19 ± 0.14	1.08 ± 0.16*	1.07 ± 0.16*	1.15 ± 0.17*
Hindlimb		1.03 ± 0.19	0.91 ± 0.14	0.90 ± 0.16	0.83 ± 0.11	0.87 ± 0.13
Forelimb	13	1.37 ± 0.18	1.24 ± 0.23	1.07 ± 0.24*	1.08 ± 0.21*	1.21 ± 0.14*
Hindlimb		1.16 ± 0.16	0.95 ± 0.18**	0.96 ± 0.19**	0.87 ± 0.11**	0.93 ± 0.12**
		Historical control data: six studies (60 males)				
Forelimb	8	Mean 1.19 (minimum 1.10; maximum 1.24)				
Hindlimb		Mean 1.02 (minimum 0.85; maximum 1.16)				
Forelimb	12/13	Mean 1.20 (minimum 1.04; maximum 1.35)				
Hindlimb		Mean 1.06 (minimum 0.89; maximum 1.17)				
Females						
Forelimb	2	0.97 ± 0.11	0.96 ± 0.11	0.87 ± 0.12	0.94 ± 0.09	0.94 ± 0.07
Hindlimb		0.66 ± 0.10	0.70 ± 0.12	0.60 ± 0.08	0.66 ± 0.09	0.62 ± 0.08
Forelimb	4	0.97 ± 0.15	1.01 ± 0.16	0.87 ± 0.17	1.01 ± 0.15	0.92 ± 0.09
Hindlimb		0.77 ± 0.17	0.77 ± 0.14	0.68 ± 0.16	0.76 ± 0.13	0.71 ± 0.11
Forelimb	8	0.99 ± 0.20	1.06 ± 0.17	0.90 ± 0.19	1.07 ± 0.14	0.90 ± 0.14
Hindlimb		0.82 ± 0.14	0.81 ± 0.22	0.74 ± 0.16	0.85 ± 0.16	0.80 ± 0.09
Forelimb	13	0.99 ± 0.31	1.06 ± 0.18	0.86 ± 0.22	1.10 ± 0.18	0.90 ± 0.20
Hindlimb		0.88 ± 0.19	0.96 ± 0.17	0.81 ± 0.15	0.87 ± 0.17	0.79 ± 0.13
		Historical control data: six studies (60 females)				
Forelimb	8	Mean 0.94 (minimum 0.89; maximum 1.03)				
Hindlimb		Mean 0.80 (minimum 0.71; maximum 0.92)				
Forelimb	12/13	Mean 0.98 (minimum 0.88; maximum 1.10)				
Hindlimb		Mean 0.87 (minimum 0.76; maximum 0.95)				

From Groom (2008b)
* $P < 0.05$; ** $P < 0.01$

decreased pup weight gain occurred at 300 mg/kg bw per day, but without effect on viability (Stannard, 2009a).

In a developmental neurotoxicity study, penthiopyrad (purity 99.2%) was administered to mated Crl:CD (SD) IGS BR female rats (22 per dose) via gavage at a dose of 0, 100, 250 or 500 mg/kg bw per day from GD 6 to LD 6, inclusive, and their offspring were dosed from PND 7 to PND day 20 or 21, inclusive. A similar control group received the vehicle only for the same period. A FOB of behavioural assessments was performed periodically on both the parent females (12 per group) and their offspring (up to 22 of each sex per group). Quantitative assessments of motor activity, sensory function, and learning and memory were also performed on the offspring. Groups of 10 male and

Table 88. Motor activity: group scores for high beam breaks (rearing activity) during week 13 of treatment in male rats

Interval (min)	Group mean scores ± SD					Historical controls[a]: mean (minimum–maximum)
	Dose (mg/kg bw per day)					
	0	10	40	160	640	
6	63.9 ± 25.5	95.7 ± 24.0*	78.0 ± 26.3	74.3 ± 21.2	60.9 ± 29.3	62.8 (34.6–91.6)
12	65.9 ± 21.2	84.6 ± 27.6	72.0 ± 12.7	59.9 ± 23.9	60.7 ± 12.9	47.0 (38.5–53.8)
18	54.6 ± 16.7	58.7 ± 21.8	44.4 ± 21.1	36.4 ± 16.8*	35.8 ± 17.2*	31.9 (21.1–46.2)
24	42.4 ± 21.1	48.8 ± 16.2	31.2 ± 13.0	32.9 ± 19.5	21.4 ± 13.4*	18.1 (7.3–29.0)
30	35.3 ± 33.2	40.5 ± 26.8	28.0 ± 13.4	22.5 ± 22.6	16.8 ± 22.0	11.0 (1.7–22.5)
36	32.8 ± 18.2	36.9 ± 18.3	23.5 ± 18.5	9.5 ± 14.4*	19.1 ± 20.5*	8.9 (3.6–15.1)
42	22.5 ± 28.5	29.8 ± 36.7	10.4 ± 13.0	7.2 ± 10.6	12.8 ± 13.2	9.1 (3.1–36.9)
48	22.6 ± 28.2	17.2 ± 19.6	9.9 ± 11.8	16.1 ± 27.5	6.5 ± 7.7	9.3 (2.8–15.1)
54	19.1 ± 19.0	14.3 ± 18.7	5.0 ± 9.6	13.0 ± 17.1	13.5 ± 16.4	7.2 (0.6–9.8)
60	12.5 ± 14.7	7.1 ± 12.1	4.8 ± 8.4	6.6 ± 10.1	10.3 ± 11.7	7.2 (3.4–8.7)
Total	371.6 ± 112.8	433.6 ± 139.2	307.2 ± 61.8	278.4 ± 83.6*	257.8 ± 52.2*	212.3 (168.6–289.9)

From Groom (2008b)
* $P \leq 0.05$
[a] Historical control values based on nine studies, 10 animals per study.

10 female offspring per group were perfused on day 21 or 66 of age for detailed neuropathological evaluation. Assessments of clinical condition, body weight performance, feed consumption, gestation length and parturition observations were also conducted on the parental females. Clinical condition, litter size and survival, sex ratio, body weight and sexual maturation were also assessed for the litters/offspring.

Oral treatment of pregnant and lactating female rats with penthiopyrad was well tolerated, and there were no treatment-related adverse findings, behavioural effects or reproductive effects at any dose level (Tables 89 and 90). At 500 or 250 mg/kg bw per day, mean offspring body weights on day 1 of age were marginally lower than those of controls, and subsequent body weight gain to day 4 of age was low in both sexes at 500 mg/kg bw per day and in males at 250 mg/kg bw per day (Table 91). Following the start of offspring treatment on day 7 of age, mean body weight gain of both sexes in the 500 and 250 mg/kg bw per day groups was lower than those of controls to scheduled termination (Table 92); however, there was no effect on mortality (Table 93). In addition, signs of perianal staining were observed for many offspring receiving 500 or 250 mg/kg bw per day. Effects on the FOB (Table 94) were confined to offspring receiving 500 mg/kg bw per day, in which a treatment-related increased incidence of occasional slight whole-body tremors was apparent at day 21 of age, but not subsequently after discontinuation of treatment. Motor activity (rearing and cage floor activity) for males at 500 and 250 mg/kg bw per day and females at 500 mg/kg bw per day was high relative to the controls at day 17 of age, but not subsequently at day 22 or 59 of age. Motor activity scores for females receiving 250 mg/kg bw per day and for males and females receiving 100 mg/kg bw per day were unaffected by treatment at all testing intervals (Table 95). There was no effect of treatment on auditory startle response pre-pulse inhibition (Table 96), but peak startle amplitude values with and without a pre-pulse in female offspring in the 500 mg/kg bw per day group were significantly lower than those of the controls at day 61/62 of age. There was no effect of treatment at any dose level on the learning and memory capacity of the offspring, as assessed by the swimming maze. Sexual maturation, assessed by the time of vaginal opening or balano-preputial separation, was unaffected by treatment at all dose levels.

Table 89. Maternal group mean feed consumption during lactation in female rats

Dose (mg/kg bw per day)	Group mean feed consumption (g/day) (± standard deviation)					
	Lactation days					
	1–3	4–6	7–10	11–13	14–16	17–20
0	44 ± 3.7	51 ± 4.5	63 ± 6.7	74 ± 6.7	81 ± 17.2	79 ± 9.1
100	43 ± 4.9	51 ± 4.9	62 ± 5.3	74 ± 6.9	79 ± 6.9	79 ± 6.4
250	42 ± 6.9	47 ± 4.6* (↓8)[a]	55 ± 4.5** (↓12)	67 ± 5.9** (↓9)	72 ± 4.8** (↓11)	74 ± 6.4
500	41 ± 4.6* (↓7)	48 ± 4.0* (↓6)	52 ± 5.4** (↓17)	71 ± 22.1** (↓4)	73 ± 7.5** (↓10)	75 ± 8.2

From Stannard (2009b). Mean data found in table 6 on p. 94 of main study.
* $P < 0.05$; ** $P < 0.01$
[a] Per cent change compared with control group.

Table 90. Summary of rat litter data and survival to day 4

Parameter	Dose (mg/kg bw per day)			
	0	100	250	500
No. mated / no. pregnant	22 / 22	22 / 22	22 / 22	22 / 22
No. with live litters	21[a]	20[b]	22	22
No. with total litter loss	0	0	0	0
No. with weaned progeny	21	20	22	22
Mean no. of implantations (± SD)	16.7 ± 1.8	17.0 ± 1.7	16.5 ± 2.1	16.0 ± 1.8
Mean total litter size on day 1 (± SD)	15.3 ± 2.5	16.1 ± 2.3	15.9 ± 1.7	14.8 ± 2.0
Mean live litter size on day (mean ± SD):				
- 1	15.0 ± 2.5	15.8 ± 2.3	15.9 ± 1.7	14.5 ± 2.2
- 4 (pre-cull)	14.9 ± 2.6	15.5 ± 2.4	15.5 ± 1.6	14.5 ± 2.2
- 4 (post-cull)	8.0 ± 0.2	8.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0
Gestation index[c] (%)	95	91	100	100
Post-implantation survival index[d] (%)	91.7	94.3	96.7	92.9
Live birth index[e] (%)	97.9	98.2	100	98.0
Viability index[f] (%)	99.3	98.0	98.1	100
Sex ratio (% males) on day (mean ± SD):				
- 1	43.8 ± 12.8	54.1 ± 16.3*	47.7 ± 14.5	53.0 ± 14.1*
- 4 (pre-cull)	44.0 ± 12.7	54.1 ± 16.2*	48.4 ± 14.4	53.0 ± 14.1
- 4 (post-cull)	49.1 ± 4.2	53.1 ± 9.0	50.0 ± 0.0	50.0 ± 3.9

From Stannard (2009b)
SD, standard deviation; * $P < 0.05$
[a] One animal with dystocia sacrificed prematurely.
[b] Two animals with dystocia sacrificed prematurely.
[c] Gestation index = (no. of live litters born/no. pregnant) × 100.
[d] Post-implantation survival index = (no. of offspring born/no. of uterine implantation sites) × 100.
[e] Live birth index = (no. of offspring live on PND 1/no. of offspring born) × 100.
[f] Viability index = (no. of live offspring on PND 4 pre-culling/no. of live offspring on PND 1) × 100.

Table 91. Group mean body weight of rat offspring prior to commencement of direct treatment

Dose (mg/kg bw per day)	Group mean body weight (g) (± standard deviation)					
	Lactation day					
	1	4 (pre-cull)	4 (post-cull)	7	14	21
Males						
0	7.0 ± 0.7	10.0 ± 1.2	10.1 ± 1.2	16.7 ± 1.9	35.2 ± 3.1	55.5 ± 4.5
100	6.8 ± 0.4	9.6 ± 0.9	9.6 ± 0.8	16.1 ± 1.5** (↓4)[a]	34.2 ± 3.1* (↓3)	55.7 ± 5.1
250	6.6 ± 0.7	9.1 ± 1.3* (↓9)	9.2 ± 1.2* (↓9)	15.3 ± 2.4** (↓8)	31.6 ± 4.9** (↓10)	51.1 ± 7.7** (↓8)
500	6.7 ± 0.6	9.2 ± 1.2* (↓8)	9.3 ± 1.1* (↓8)	15.5 ± 1.5** (↓7)	28.9 ± 3.7** (↓18)	48.4 ± 5.4** (↓13)
Females						
0	6.7 ± 0.6	9.6 ± 1.2	9.5 ± 1.3	15.8 ± 1.8	33.6 ± 3.2	53.3 ± 4.5
100	6.5 ± 0.4	9.0 ± 0.8	9.0 ± 0.8	15.1 ± 1.3** (↓4)	32.6 ± 3.3 (↓3)	52.8 ± 5.0 (↓4)
250	6.3 ± 0.6* (↓6)	8.8 ± 1.0* (↓3)	8.9 ± 1.0 (↓6)	14.9 ± 1.9** (↓6)	31.2 ± 3.7** (↓7)	50.0 ± 5.8 (↓4)
500	6.3 ± 0.5* (↓6)	8.7 ± 1.1 (↓4)	8.7 ± 1.2* (↓8)	14.7 ± 1.8** (↓7)	27.3 ± 4.3** (↓19)	46.9 ± 6.0** (↓12)

From Stannard (2009b)
* $P < 0.05$; ** $P < 0.01$
[a] Per cent change compared with control group.

Table 92. Group mean body weight gain of rat offspring from commencement of treatment to day 63

Dose (mg/kg bw per day)	Mean body weight (g) (± SD)	Mean body weight gain (g) (± SD)			
	Day 7	Days 7–13	Days 13–21	Days 21–35	Days 35–63
Males					
0	16.7 ± 1.9	15.9 ± 1.7	22.9 ± 2.3	99 ± 11	248 ± 25
100	16.1 ± 1.5** (↓4)[a]	15.6 ± 2.0	24.0 ± 2.9	96 ± 8	246 ± 20
250	15.3 ± 2.4** (↓8)	13.7 ± 2.6** (↓14)	22.0 ± 2.3	96 ± 9	244 ± 23
500	15.5 ± 1.5** (↓7)	10.8 ± 2.5** (↓32)	22.1 ± 2.5	91 ± 13	241 ± 28
Females					
0	15.8 ± 1.8	15.3 ± 2.0	22.2 ± 2.7	79 ± 8	114 ± 16
100	15.1 ± 1.3** (↓4)	15.0 ± 2.4	22.6 ± 3.0	77 ± 8	118 ± 14
250	14.9 ± 1.9** (↓6)	13.9 ± 1.9** (↓9)	21.3 ± 3.0	77 ± 8	114 ± 17
500	14.7 ± 1.8** (↓7)	10.2 ± 2.7** (↓33)	22.0 ± 2.7	73 ± 7	116 ± 14

From Stannard (2009b)
SD, standard deviation; ** $P < 0.01$
[a] Per cent change compared with control group.

Table 93. Rat offspring mortality prior to dosing on postnatal days 7–21

	0 mg/kg bw per day	100 mg/kg bw per day	250 mg/kg bw per day	500 mg/kg bw per day
Males				
Group size	82	84	86	88
Number of deaths	4	4	4	3
Deaths due to dosing trauma	3	4	3	1

Table 93 (continued)

	0 mg/kg bw per day	100 mg/kg bw per day	250 mg/kg bw per day	500 mg/kg bw per day
Deaths with no evidence of dosing trauma	1	0	1	2
Females				
Group size	84	75	87	88
Number of deaths	5	1	6	8
Deaths due to dosing trauma	0	1	4	3
Deaths with no evidence of dosing trauma	5	0	2	5

From Stannard (2009b)

Table 94. Selected functional observational battery observations on rat offspring in the arena

Observation interval	Sex	Dose (mg/kg bw per day)	Group value	
			No. with whole-body tremor/no. tested	Mean activity score (sector entries)
Day 4	Male	0	0/21	2.0
		100	0/20	1.9
		250	0/22	1.4
		500	0/22	1.4
	Female	0	0/21	1.9
		100	0/20	1.5
		250	0/22	1.7
		500	0/22	1.4
Day 11	Male	0	0/21	6.1
		100	0/20	6.5
		250	0/22	7.1
		500	0/22	8.1
	Female	0	0/21	8.3
		100	0/20	7.9
		250	0/22	9.4
		500	0/22	7.7
Day 21	Male	0	0/21	7.4 ± 5.1[a]
		100	1/20	7.3 ± 4.2
		250	2/22	6.3 ± 3.8
		500	5/22	8.7 ± 6.6
	Female	0	1/21	7.0 ± 5.6
		100	0/20	7.8 ± 4.7
		250	0/22	6.0 ± 5.0
		500	5/22	7.6 ± 5.6
Day 35	Male	0	1/21	13.3 ± 5.3
		100	2/20	9.6 ± 6.4
		250	2/22	12.0 ± 6.5
		500	1/22	15.4 ± 6.9

Table 94 (continued)

Observation interval	Sex	Dose (mg/kg bw per day)	Group value	
			No. with whole-body tremor/no. tested	Mean activity score (sector entries)
	Female	0	0/21	13.3 ± 6.3
		100	0/20	12.4 ± 6.1
		250	1/22	11.9 ± 8.8
		500	0/22	17.3 ± 5.5
Day 45	Male	0	1/21	14.4 ± 6.1
		100	0/20	11.7 ± 7.9
		250	0/22	14.4 ± 7.3
		500	0/22	14.5 ± 7.3
	Female	0	0/21	21.0 ± 7.7
		100	0/20	20.4 ± 8.2
		250	1/22	21.5 ± 8.4
		500	0/22	23.2 ± 7.5
Day 60	Male	0	0/21	14.6 ± 7.0
		100	0/20	17.0 ± 7.8
		250	0/22	16.7 ± 7.5
		500	1/22	19.2 ± 7.0*
	Female	0	0/21	26.2 ± 5.1
		100	0/20	24.3 ± 7.5
		250	0/22	27.3 ± 7.5
		500	0/22	27.6 ± 4.8

From Stannard (2009b)
* $P < 0.05$
[a] Standard deviation.

Table 95. Group mean quantitative motor activity scores on rat offspring

Observation interval	Sex	Dose (mg/kg bw per day)	Group mean total scores ± standard deviation	
			High beam breaks (rearing activity)	Low beam breaks (cage floor activity)
Day 13	Male	0	14.8 ± 22.4	623.0 ± 445.9
		100	14.1 ± 22.7	566.5 ± 458.9
		250	26.5 ± 37.8	489.4 ± 280.0
		500	23.3 ± 44.2	755.5 ± 470.8
		HCD[a]: minimum–maximum (mean)	9.5–17.8[b] (13.8)	326.4–493.1 (406.2)
	Female	0	25.9 ± 28.0	570.1 ± 427.5
		100	21.1 ± 36.5	684.7 ± 355.9
		250	16.8 ± 33.3	470.7 ± 291.9
		500	38.1 ± 42.7	717.3 ± 429.9
		HCD[a]: minimum–maximum (mean)	6.5–25.8 (17.8)	173.4–633.1 (448.4)

Table 95 (continued)

Observation interval	Sex	Dose (mg/kg bw per day)	Group mean total scores ± standard deviation	
			High beam breaks (rearing activity)	Low beam breaks (cage floor activity)
Day 17	Male	0	119.6 ± 137.6	684.4 ± 485.8
		100	113.7 ± 120.8	925.2 ± 888.1
		250	203.8 ± 156.3	1109.0 ± 676.7
		500	220.2 ± 198.1*	1344.9 ± 818.2**
		HCD[a]: minimum–maximum (mean)	35.4–130.2 (72.0)	317.8–1165.3 (742.2)
	Female	0	158.0 ± 147.4	932.2 ± 626.4
		100	175.8 ± 178.2	906.2 ± 714.6
		250	185.3 ± 162.4	971.4 ± 653.7
		500	225.8 ± 194.9	1332.3 ± 809.0
		HCD[a]: minimum–maximum (mean)	105.8–243.3 (151.1)	830.7–1421.3 (1048.5)
Day 22	Male	0	183.1 ± 104.1	472.5 ± 250.9
		100	196.4 ± 133.0	456.8 ± 269.4
		250	177.5 ± 74.0	502.8 ± 208.8
		500	236.0 ± 162.4	618.7 ± 382.3
		HCD[a]: minimum–maximum (mean)	102.7–259.4 (155.8)	326.9–729.5 (468.0)
	Female	0	152.9 ± 110.0	386.5 ± 204.9
		100	183.3 ± 111.1	493.8 ± 241.7
		250	160.4 ± 98.7	482.1 ± 235.3
		500	194.1 ± 172.5	527.4 ± 356.4
		HCD[a]: minimum–maximum (mean)	97.8–182.5 (142.9)	379.7–492.9 (447.0)
Day 59	Male	0	492.6 ± 149.4	1205.2 ± 270.2
		100	428.2 ± 128.8	1240.6 ± 270.1
		250	484.2 ± 180.9	1230.6 ± 311.9
		500	534.7 ± 174.6	1270.1 ± 306.3
		HCD: minimum–maximum (mean)	364.5–649.7 (461.5)	1150.3–1652.8 (1365.7)
	Female	0	550.9 ± 171.2	1270.8 ± 363.0
		100	729.3 ± 262.8*	1326.7 ± 436.8
		250	806.5 ± 334.8**	1514.1 ± 331.6
		500	767.8 ± 319.1**	1452.8 ± 382.1
		HCD[a]: minimum–maximum (mean)	391.6–813.5 (589.9)	1135.9–1718.8 (1392.9)

From Stannard (2009b)

HCD, historical control data; * $P < 0.05$; ** $P < 0.01$

[a] Historical control data derived from four studies from February 2004 to September 2007. Route of administration (gavage versus dietary) was not specified for historical control data.

[b] Range of total 1-hour scores.

Table 96. Summary of auditory startle response pre-pulse inhibition in rat offspring

Age (days) and sex	Dose (mg/kg bw per day)	Group mean value ± standard deviation				
		Latency to peak (ms)		Peak amplitude (g)		
		− pre-pulse	+ pre-pulse	− pre-pulse	+ pre-pulse	% inhibition
23/24 Males	0	14.2 ± 4.4	14.2 ± 1.3	153.0 ± 32.2	122.9 ± 25.0	19.2 ± 7.0
	100	13.3 ± 1.0	13.8 ± 1.7	160.2 ± 27.5	129.4 ± 25.1	19.2 ± 7.7
	250	13.0 ± 0.9	14.0 ± 1.6	148.6 ± 29.3	116.4 ± 22.7	21.1 ± 7.7
	500	14.1 ± 2.2	14.0 ± 1.5	129.2 ± 31.3*	103.2 ± 24.3*	19.3 ± 10.6
23/24 Females	0	13.6 ± 1.9	13.8 ± 1.3	147.4 ± 26.8	116.4 ± 17.3	20.1 ± 9.2
	100	13.6 ± 1.7	13.8 ± 1.5	146.0 ± 27.1	119.0 ± 24.9	18.7 ± 5.7
	250	13.3 ± 1.7	13.9 ± 1.6	135.3 ± 29.3	107.0 ± 21.0	19.9 ± 8.6
	500	14.1 ± 2.3	14.5 ± 1.5	130.1 ± 28.5	99.6 ± 20.3*	22.7 ± 8.3
61/62 Males	0	15.3 ± 3.2	16.5 ± 3.3	754.8 ± 162.0	572.5 ± 120.4	23.4 ± 8.8
	100	16.3 ± 4.7	16.8 ± 3.4	804.8 ± 234.5	591.3 ± 120.4	24.9 ± 8.0
	250	15.0 ± 3.9	16.9 ± 4.9	757.9 ± 190.2	549.1 ± 93.7	25.2 ± 12.8
	500	14.4 ± 2.4	17.5 ± 5.2	774.3 ± 226.1	549.7 ± 147.7	27.1 ± 13.2
61/62 Females	0	16.4 ± 4.3	18.6 ± 4.8	535.5 ± 179.8	370.9 ± 84.1	28.4 ± 11.2
	100	16.7 ± 4.9	17.0 ± 3.5	521.0 ± 168.8	394.1 ± 99.3	22.2 ± 11.8
	250	18.4 ± 6.4	19.7 ± 6.8	461.5 ± 107.2	334.0 ± 60.9	26.4 ± 9.7
	500	17.9 ± 5.3	19.9 ± 5.8	428.5 ± 128.3*	316.5 ± 61.3*	23.7 ± 11.4

From Stannard (2009b)
* $P < 0.05$

Table 97. Summary of brain dimensions in day 66 perfused rat offspring

Dose (mg/kg bw per day)	Group mean value (mm) ± standard deviation			
	Males		Females	
	Length	Width	Length	Width
0	21.3 ± 0.4	15.5 ± 0.4	20.5 ± 0.3	15.2 ± 0.4
100	21.1 ± 0.4	15.5 ± 0.3	20.5 ± 0.4	15.1 ± 0.3
250	20.9 ± 0.4*	15.3 ± 0.4	20.4 ± 0.7	15.2 ± 0.3
500	20.9 ± 0.3*	15.3 ± 0.2	20.4 ± 0.4	15.0 ± 0.2

From Stannard (2009b)
* $P < 0.05$

There were no treatment-related macroscopic findings in the offspring at scheduled termination, no effects on brain weight, no changes in brain morphometry at day 21 or day 66 of age (Table 97) and no histopathological changes in the tissues of the central and peripheral nervous systems presented for neuropathological examination on day 21 or day 66 of age (Table 98).

The maternal NOAEL was 500 mg/kg bw per day, based on the absence of maternal adverse effects up to the highest dose tested. The NOAEL for the F_1 offspring was 100 mg/kg bw per day, based on decreased body weights up to PND 7 observed at 250 mg/kg bw per day (Stannard, 2009b).

Table 98. Summary incidence of histopathological findings (degenerate fibres) in peripheral nerves of rat offspring

Tissue	Incidence of degenerate fibres / no. examined			
	Males		Females	
	Dose (mg/kg bw per day)			
	0	500	0	500
Sciatic nerve (thigh)	4/9	4/10	3/10	5/10
Sciatic nerve (notch)	7/9	7/10	3/10	4/10
Tibial nerve (knee)	3/9	3/10	2/10	2/10
Tibial nerve (calf)	4/9	4/10	2/10	4/10

From Stannard (2009b)

(c) Immunotoxicity

In an immunotoxicity study, penthiopyrad (purity 98.6%) was administered in the diet to SD CD-1 mice (10 males per dose) at a target dose level of 0, 62.5, 250 or 1000 mg/kg bw per day for 4 weeks. A similar group of mice, given daily oral (gavage) doses of cyclophosphamide at 20 mg/kg bw per day for 5 days (from 7 to 3 days before termination), acted as a positive control group. All animals received a sensitizing intravenous dose of sheep red blood cells in 0.9% saline 4 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 detailed necropsy after 4 weeks of treatment, and the weights of brain, liver, spleen and thymus were recorded. Bone marrow smears were prepared and examined from all control and high-dose animals. A semiquantitative histopathological assessment of lymphoid tissue compartments, with respect to both the lymphocyte and non-lymphocyte components, was performed on thymus, lymph nodes, spleen, mucosa-associated lymphoid tissue and bone marrow from the control and all penthiopyrad-treated animals. All splenic tissue not required for histology from all test, control and positive control animals was used as a source of splenocytes for assessment of the adaptive or acquired immune response to the T cell–dependent immunogen, sheep red blood cells, using a modification of the Jerne plaque-forming cell (PFC) assay. The number of lytic plaques for each animal was determined, and group mean responses were calculated and expressed as group mean number of PFCs per spleen and per 10^6 splenocytes.

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. Overall water intake was increased by 20% and 26% at 250 and 1000 mg/kg bw per day, but this was considered not to be an adverse effect.

There were no treatment-related changes in total or differential white blood cell counts at any dose level or in the positive control group. There were no treatment-related gross lesions identified at necropsy. Absolute and body weight–adjusted liver weights were slightly higher than control values at 250 and 1000 mg/kg bw per day, but not at the lowest dose level. There were no effects of treatment on the weights of spleen and thymus. There were no treatment-related changes in the cellularity, distribution or morphology of bone marrow cells at 1000 mg/kg bw per day, and treatment-related histopathological changes were confined to the spleen of animals treated at 1000 mg/kg bw per day. There was an increased incidence of minimal or slight extramedullary haematopoiesis, which was considered not to be an adverse effect of treatment, and a higher incidence of increased cellularity/size of the periarteriolar lymphocyte sheath at 1000 mg/kg bw per day (Table 99). The latter finding was considered to be of uncertain relationship to treatment and is also considered not to be an adverse

Table 99. Selected histopathological alterations of the spleen of mice

Finding	Severity grade	Incidence			
		Dose (mg/kg bw per day)			
		0	62.5	250	1000
Number of animals examined		*10*	*10*	*10*	*10*
Red pulp, extramedullary haematopoiesis	Minimal	1	4	6	6
	Slight	4	3	1	4
Increased cellularity/size of periarteriolar lymphocyte sheath	Minimal	2	1	3	6

From Webley (2009)

Table 100. Group mean PFC assay data in mice

Dose (mg/kg bw per day)	Group mean value ± standard deviation (n = 10)		
	Cells/spleen ($\times 10^7$)	PFCs/10^6 cells	PFCs/spleen
0	10.95 ± 3.18	2728.5 ± 1107.9	306 489 ± 157 994
62.5	10.56 ± 2.79	2701.5 ± 1023.6	291 590 ± 150 433
250	11.05 ± 3.81	2914.0 ± 1398.8	324 777 ± 189 104
1000	10.62 ± 2.97	1223.5 ± 467.0***	124 437 ± 44 551**
Cyclophosphamide (20)	5.93 ± 2.42***	54.3 ± 29.7***	3491 ± 3539***

From Webley (2009)
PFC, plaque-forming cell; ** $P < 0.01$; *** $P < 0.001$

response to treatment, but rather a functional change suggesting an expansion of T lymphocyte numbers. There were no other treatment-related changes identified during the semiquantitative assessment of lymphoid tissues with respect to both lymphocyte and non-lymphocyte components.

There was a statistically significant decrease in the numbers of PFCs, expressed both as PFCs per spleen and as PFCs per 10^6 splenocytes at 1000 mg/kg bw per day, but not at lower dose levels (Table 100). However, there was no histopathological evidence of a decrease in splenic B-cell numbers. Treatment with cyclophosphamide resulted in statistically significant decreases in the numbers of plaques per spleen and per 10^6 cells and the number of cells per spleen, demonstrating the sensitivity of the test system.

The NOAEL for immunotoxicity and systemic effects was 250 mg/kg bw per day, based on a decrease in plaque-forming ability observed at 1000 mg/kg bw per day (Webley, 2009).

In an immunotoxicity study, penthiopyrad (purity 98.6%) was administered in the diet to Sprague-Dawley rats (10 males per dose) at a target dose level of 0, 45, 170 or 700 mg/kg bw per day for 4 weeks. A similar group of rats, given a single intraperitoneal injection of cyclophosphamide at 50 mg/kg bw 2 days before termination, acted as a positive control group. All animals received a sensitizing intravenous dose of sheep red blood cells in 0.9% saline 4 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 taken on day 28/29. All animals were subjected to detailed necropsy after 4 weeks of treatment, and the weights of brain, liver, spleen, thymus and lymph nodes from three locations were recorded. Bone marrow smears were prepared and examined from all control and high-dose animals. A semiquantitative histopathological assessment of lymphoid tissue compartments, with respect to both the lymphocyte and non-lymphocyte components, was performed on

thymus, lymph nodes, spleen, mucosa-associated lymphoid tissue and bone marrow from the control and all penthiopyrad-treated animals. All splenic tissue not required for histology from all test, control and positive control animals was used as a source of splenocytes for assessment of the adaptive or acquired immune response to the T cell–dependent immunogen, sheep red blood cells, using a modification of the Jerne PFC assay. The number of lytic plaques for each animal was determined, and group mean responses were calculated and expressed as group mean number of PFCs per spleen and per 10^6 splenocytes.

All animals survived the scheduled treatment period, and there were no treatment-related clinical signs. Body weight loss and reduced feed consumption, followed by reduced weight gain, occurred at 700 mg/kg bw per day, but there was no adverse effect on weight gain or feed consumption at lower dose levels or in the positive control group (Table 101). Water intake was increased at 700 mg/kg bw per day, possibly related to diet palatability. There were no treatment-related changes in total or differential white blood cell counts at any dose level or in the positive control group. Treatment-related gross lesions identified at necropsy were confined to liver enlargement, correlated with increased weight, at 700 mg/kg bw per day, but not at lower dose levels. Absolute and relative spleen weights were significantly lower than control values at 700 mg/kg bw per day, but not at lower dose levels. The absolute and relative weights of the spleen, thymus and all weighed lymph nodes were lower than control values in the cyclophosphamide positive control group (Table 102). There was no effect of treatment with penthiopyrad at any dose level on the weights of lymph nodes and thymus. There were no treatment-related changes in the cellularity, distribution or morphology of bone marrow cells at 700 mg/kg bw per day, and treatment-related histopathological changes were confined to the thymus. A dose-related increase in the incidence of minimal thymic cortical tingible body macrophages occurred at 175 and 700 mg/kg bw per day. This change was attributed to engulfed nuclear material from B lymphocytes and was considered not to be an adverse effect of treatment or evidence of immunotoxicity. There were no treatment-related changes identified during the semiquantitative assessment of lymphoid tissue components with respect to both lymphocyte and non-lymphocyte components. There was a significant decrease in the number of cells recovered from the spleen of animals at 700 mg/kg bw per day, and there was a collateral, non-significant decrease in the total number of PFCs per spleen at 700 mg/kg bw per day. However, there was no effect of treatment at any dose level on the number of PFCs per 10^6 spleen cells (Table 103). As there was no effect of treatment on the functional assessment of the humoral immune response to a T lymphocyte–dependent antigen, and in the absence of any other treatment-related changes in haematology or histopathology of the lymphatic tissues, the decrease in total spleen cell numbers at 700 mg/kg bw per day was considered not to be of toxicological significance. The mean numbers of cells per spleen, PFCs per 10^6 cells and PFCs per spleen at 45 or 175 mg/kg bw per day were comparable to, or greater than, the control values. The PFC response for the animals treated with cyclophosphamide was significantly ablated. The cyclophosphamide-treated group also showed a marked reduction in the total number of spleen cells.

The NOAEL for immunotoxic effects was 700 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 maximum tolerated dose of 700 mg/kg bw per day. A LOAEL for immunotoxicity was not observed.

The NOAEL for systemic effects was 175 mg/kg bw per day, based on the occurrence of reduced body weight gain and feed consumption, increased liver weight and a secondary reduction in spleen weight at 700 mg/kg bw per day (Kilpatrick, 2009).

(d) Studies on metabolites

The acute and short-term oral toxicity and the genotoxic potential of the metabolites DM-PCA and PCA and the acute toxicity and genotoxic potential of the metabolites PAM, 753-A-OH and 753-T-DO were investigated. The studies were reported to comply with GLP and performed according to internationally accepted guidelines.

Table 101. Selected group mean body weight and body weight change data in rats

Dose (mg/kg bw per day)	Group mean body weight (g) ± standard deviation (n =10)				Group mean body weight change (g) ± standard deviation		
	Day(s)						
	1	8	22	29	1–8	8–29	1–29
0	227 ± 17.7	285 ± 23.6	365 ± 34.1	389 ± 36.4	58 ± 6.8	104 ± 17.9	162 ± 23.4
45	233 ± 14.6	283 ± 17.6	366 ± 21.5	385 ± 22.8	50 ± 7.0**	102 ± 10.2	152 ± 16.0
175	235 ± 12.0	284 ± 16.4	361 ± 32.5	388 ± 40.9	49 ± 6.8**	104 ± 28.1	153 ± 34.0
700	239 ± 17.4	250 ± 19.2	326 ± 30.1	345 ± 33.6	11 ± 4.9**	95 ± 19.6	106 ± 18.8**
Cyclophosphamide (50)	226 ± 10.2	286 ± 14.7	371 ± 24.0	394 ± 28.2	60 ± 6.2	108 ± 20.7	168 ± 23.2

From Kilpatrick (2009)
** $P < 0.01$

Table 102. Selected organ weight data in rats

Dose (mg/kg bw per day)	Mean body weight (g)	Group mean organ weight ± standard deviation (n = 10)					
		Brain		Liver		Spleen	
		g	% body weight	g	% body weight	g	% body weight
0	397 ± 38	1.97 ± 0.07	0.498 ± 0.039	17.89 ± 2.81	4.50 ± 0.42	0.794 ± 0.12	0.202 ± 0.037
45	391 ± 26	1.97 ± 0.07	0.506 ± 0.026	17.77 ± 2.07	4.55 ± 0.42	0.796 ± 0.06	0.204 ± 0.018
175	390 ± 41	1.95 ± 0.11	0.504 ± 0.047	18.70 ± 2.02	4.81 ± 0.33	0.764 ± 0.16	0.195 ± 0.031
700	344 ± 33**	1.94 ± 0.11	0.567 ± 0.042**	19.30 ± 2.43	5.60 ± 0.43**	0.572 ± 0.08**	0.168 ± 0.031*
Cyclophosphamide (50)[a]	402 ± 27	1.97 ± 0.07	0.492 ± 0.036	19.25 ± 2.32	4.79 ± 0.34	0.472 ± 0.069	0.118 ± 0.016

From Kilpatrick (2009)
* $P < 0.05$; ** $P < 0.01$
[a] Not analysed statistically.

Table 103. Group mean plaque-forming cell assay data in rats

Dose (mg/kg bw per day)	Group mean ± standard deviation (n = 10)		
	Cells/spleen ($\times 10^7$)	PFCs/10^6 cells	PFCs/spleen
0	50.67 ± 13.94	1455.8 ± 684.1	738 975 ± 390 002
45	55.48 ± 11.11	2206.5 ± 1243.6	1 175 626 ± 637 346
175	45.74 ± 14.44	1889.5 ± 1337.2	851 428 ± 665 875
700	36.71 ± 8.67*	1595.3 ± 607.5	593 839 ± 295 384
Cyclophosphamide (50)	9.93 ± 3.85***	35.8 ± 32.7***	3658 ± 3928***

From Kilpatrick (2009)
PFC, plaque-forming cell; * $P < 0.05$; *** $P < 0.001$

Metabolite DM-PCA (3-trifluoromethyl-1H-pyrazole-4-carboxylic acid)

In an acute oral toxicity study (acute toxic class method), single oral gavage doses of DM-PCA (purity 99.71%) were administered as an aqueous methylcellulose suspension, at 10 ml/kg bw, to two groups of three food-deprived female Crl:CD Sprague-Dawley (SPF) rats at a dose level of 2000 mg/kg bw.

No mortalities or clinical signs of toxicity occurred, and 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 (Oda, 2005a).

In a 2-week range-finding study, groups of five Wistar rats of each sex per dose received DM-PCA (purity 98.1%) in the diet at doses of 0, 3200, 8000 or 20 000 ppm (equal to doses of 0, 288, 718 and 1731 mg/kg bw per day in males and 0, 310, 742 and 1785 mg/kg bw per day in females). There were no deaths and no adverse clinical signs at any dose level, other than soft faeces at 1000 mg/kg bw per day. Females showed a greater than or equal to 22% decrease in body weight gain at and above 8000 ppm associated with reduced feed consumption at 20 000 ppm. There were no other effects of treatment at any dose level (Moore, 2008).

In a subchronic toxicity study, DM-PCA (purity 98.1%) was administered in the diet to Wistar rats (10 of each sex per dose) at a concentration of 0, 1000, 4000 or 16 000 ppm for 13 weeks, equal to overall mean doses of 0, 66, 258 and 1038 mg/kg bw per day in males and 0, 77, 306 and 1200 mg/kg bw per day in females.

There were no deaths and no treatment-related adverse clinical signs or behavioural effects at any dose level. Responses to sensory reactivity tests, grip strength and motor activity measurements did not reveal treatment-related effects at any dose level (Tables 104 and 105). Effects on body weight gain were confined to males at 16 000 ppm, which showed reduced weight gain from the onset of treatment, such that in week 13, mean body weight was reduced by 13.1% and overall weight gain was 24.4% lower than that of the controls (Table 106). Males treated at 16 000 ppm showed 7% lower feed consumption than the controls, but feed consumption of females at this dose level and both sexes at lower dose levels was unaffected by treatment. There were no treatment-related ocular lesions in either sex at 16 000 ppm after 13 weeks of treatment. In week 13, both sexes at 16 000 ppm showed slightly lower haematocrit values, accompanied in males by slightly lower red blood cell and reticulocyte counts. Total white blood cell count was also significantly reduced in males at this dose level, predominantly the result of a reduced lymphocyte count. These minor differences were not apparent in females at 16 000 ppm and not considered adverse. The differences were not seen in either sex at lower dose levels (Table 107). In week 13, males treated at 16 000 ppm showed slightly higher plasma alkaline phosphatase and AST activities. Plasma calcium ion concentration in all treated male groups showed a minimal, but dose-related, decrease relative to the controls. At 1000 and 4000 ppm, the difference from the control value was less than or equal to 2.3% and was considered not toxicologically relevant. Group mean plasma phosphorus concentrations in males at 4000 and 16 000 ppm were significantly lower than the control value. Females at 16 000 ppm showed no treatment-related alterations in the plasma chemistry profile. With the exception of slightly lower phosphorus concentration in males at 4000 ppm, which in isolation was considered a non-adverse change, the plasma chemistry profiles of both sexes at lower dose levels were unaffected by treatment (Table 108). The clinical chemistry alterations in blood plasma of males at 16 000 ppm were not associated with any effect on liver and kidney weights or morphology, suggesting that the effects were minor adaptive changes related to metabolism and/or excretion. Therefore, they were not considered toxicologically relevant. Urine pH was significantly lower than the control value in females at 4000 ppm and in both sexes at 16 000 ppm, but there was no effect of treatment at any dose level on urine volume and specific gravity. This effect was not considered adverse, but possibly indicative of acidic products of metabolism. Males at 16 000 ppm also showed significantly higher protein concentration (Table 109).

There were no treatment-related macroscopic findings at necropsy and no organ weight changes at any dose level, other than some low absolute weights related to low body weight in males at 16 000 ppm. No treatment-related histopathological alterations occurred in any of the organs examined.

Table 104. Group mean grip strength in week 12 in rats

Limb	Sex	Group mean grip strength (kg) ± standard deviation			
		Dietary concentration (ppm)			
		0	1000	4000	16 000
Forelimb	Male	1.25 ± 0.18	1.15 ± 0.23	1.25 ± 0.19	1.10 ± 0.25
Hindlimb		1.05 ± 0.11	0.85 ± 0.13*	1.05 ± 0.18	0.90 ± 0.16
Forelimb	Female	1.13 ± 0.13	1.08 ± 0.11	1.05 ± 0.13	0.97 ± 0.15*
Hindlimb		0.82 ± 0.14	0.79 ± 0.10	0.88 ± 0.10	0.75 ± 0.08
Historical control data: six studies (60 males, 60 females)		Males		Females	
Forelimb	Week 12/13	Mean 1.20 (minimum 1.04, maximum 1.35)		Mean 0.98 (minimum 0.88, maximum 1.10)	
Hindlimb		Mean 1.06 (minimum 0.89, maximum 1.17)		Mean 0.87 (minimum 0.76, maximum 0.95)	

From Moore (2009)
* $P < 0.05$

Table 105. Female group mean motor activity in week 12 in rats

Interval (min)	Group mean beam score in females ± standard deviation							
	Low beam[a]				High beam			
	Dietary concentration (ppm)							
	0	1000	4000	16 000	0	1000	4000	16 000
6	225.0 ± 64.4	261.7 ± 64.8	278.3 ± 48	257.1 ± 85.8	118.2 ± 43.5	136.2 ± 53.5	125.3 ± 31.3	132.9 ± 65.7
12	121.8 ± 59.5	152.3 ± 67.9	155.4 ± 59.8	160.0 ± 61.5	50.9 ± 20.3	86.8 ± 41.1	72.8 ± 36.1	60.1 ± 54.5
18	72.2 ± 27.6	113.4 ± 47.1	134.1 ± 63.6	96.0 ± 62.9	35.8 ± 20.5	64.9 ± 38.5	54.4 ± 31.5	40.4 ± 39.9
24	66.6 ± 23.3	110.8 ± 52.9	107.9 ± 48.3	73.9 ± 53.1	27.6 ± 25.9	37.9 ± 21.0	26.2 ± 18.0	25.4 ± 27.3
30	59.8 ± 34.1	97.2 ± 57.5	68.9 ± 33	51.5 ± 22.9	20.8 ± 18.9	46.6 ± 28.4*	18.2 ± 11.5	20.3 ± 17.1
36	41.9 ± 23.2	99.5 ± 34.9	58.6 ± 43.3	71.3 ± 46.7	19.6 ± 11	32.4 ± 18.8	15.4 ± 25.4	26.3 ± 24
42	52.2 ± 23.6	63.0 ± 37.4	56.5 ± 52.0	48.8 ± 59.3	16.3 ± 19.3	15.1 ± 15.9	14.5 ± 13.4	13.8 ± 17.6
48	53.3 ± 33.9	86.2 ± 32.0	57.9 ± 49.7	41.4 ± 50.3	7.8 ± 7.6	25.4 ± 14.5	18.6 ± 20.8	18.3 ± 35.9
54	37.3 ± 42.0	118.2 ± 92.5*	68.7 ± 41.4	25.7 ± 44.5	17.9 ± 20.7	32.1 ± 30.8	23.2 ± 17.0	12.2 ± 19.8
60	49.8 ± 49.9	65.3 ± 49.4	57.9 ± 44.9	53.2 ± 58.4	14.5 ± 16.5	15.4 ± 17.5	16.1 ± 20	28.5 ± 31.7
Total (0–60)	779.9 ± 181.3	1167.6 ± 262.3*	1044.2 ± 181.3*	878.9 ± 341.3*	329.4 ± 129.9	492.8 ± 119.7	384.7 ± 110.8	378.2 ± 257.5

From Moore (2009)
* $P < 0.05$
[a] Historical control data: low beam, mean 844.7 (minimum 538.8, maximum 1279.2).

Table 106. Group mean body weight at selected intervals and overall weight gain in rats

Dietary concentration (ppm)	Group mean body weight (g) ± standard deviation					Group mean body weight gain (g) ± standard deviation
	Week(s)					
	0	2	4	8	13	0–13
Males						
0	201 ± 13.4	273 ± 21.6	323 ± 29.1	391 ± 38.9	434 ± 42.3	234 ± 33.4
1000	191 ± 18.2	253 ± 26.9	298 ± 32.5	353 ± 39.3	397 ± 41.7	206 ± 30.6

Table 106 (continued)

Dietary concentration (ppm)	Group mean body weight (g) ± standard deviation					Group mean body weight gain (g) ± standard deviation
	Week(s)					
	0	2	4	8	13	0–13
4000	203 ± 15.0	278 ± 23.6	330 ± 33.6	396 ± 45.2	437 ± 48.8	234 ± 39.3
16 000	200 ± 14.7	254 ± 25.9	292 ± 34.2	341 ± 39.5	377 ± 39.1	177 ± 31.0**
Females						
0	142 ± 6.2	168 ± 10.9	188 ± 10.5	213 ± 13.3	225 ± 15.1	83 ± 10.1
1000	145 ± 12.3	179 ± 17.2	202 ± 19.3	229 ± 20.9	242 ± 20.8	97 ± 13.7
4000	150 ± 12.7	181 ± 17.1	200 ± 19.7	224 ± 23.5	238 ± 25.9	88 ± 16.3
16 000	147 ± 8.9	173 ± 12.3	194 ± 11.1	219 ± 14.6	230 ± 15.3	83 ± 11.9

From Moore (2009)
** $P < 0.01$

Table 107. Selected group mean haematological values after 13 weeks of treatment in rats

Parameter (unit)	Group mean value ± standard deviation			
	Dietary concentration (ppm)			
	0	1000	4000	16 000
Males				
Haematocrit (l/l)	0.458 ± 0.01	0.449 ± 0.02	0.450 ± 0.01	0.440 ± 0.02*
Red blood cells (× 10^{12}/l)	8.80 ± 0.23	8.66 ± 0.36	8.86 ± 0.46	8.58 ± 0.35
Reticulocytes (%)	2.13 ± 0.22	1.96 ± 0.31	1.85 ± 0.23*	1.86 ± 0.27*
White blood cells (× 10^{9}/l)	8.56 ± 1.9	7.77 ± 1.2	7.40 ± 1.4	5.76 ± 1.2**
Females				
Haematocrit (l/l)	0.425 ± 0.01	0.410 ± 0.01	0.421 ± 0.02	0.410 ± 0.02*
Red blood cells (× 10^{12}/l)	7.90 ± 0.26	7.70 ± 0.30	7.86 ± 0.28	7.80 ± 0.52
Reticulocytes (%)	2.15 ± 0.40	2.15 ± 0.39	2.11 ± 0.31	2.12 ± 0.40
White blood cells (× 10^{9}/l)	4.00 ± 1.14	4.83 ± 0.64	4.85 ± 1.64	4.86 ± 0.91

From Moore (2009)
* $P < 0.05$; ** $P < 0.01$

Table 108. Selected group mean plasma chemistry values after 13 weeks of treatment in rats

Parameter (unit)	Group mean value ± standard deviation			
	Dietary concentration (ppm)			
	0	1000	4000	16 000
Males				
Alkaline phosphatase (U/l)	59 ± 5.0	71 ± 13.6	61 ± 6.8	69 ± 9.9*
AST (U/l)	61 ± 7.2	61 ± 6.7	61 ± 6.2	86 ± 48.3**
ALT (U/l)	33 ± 9.0	35 ± 9.3	28 ± 6.8	47 ± 43.8
Calcium (mmol/l)	2.65 ± 0.06	2.60 ± 0.08*	2.59 ± 0.34*	2.54 ± 0.05**
Phosphorus (mmol/l)	1.80 ± 0.14	1.79 ± 0.19	1.62 ± 0.11*	1.54 ± 0.16**

Table 108 (continued)

Parameter (unit)	Group mean value ± standard deviation			
	Dietary concentration (ppm)			
	0	1000	4000	16 000
Females				
Alkaline phosphatase (U/l)	29 ± 5.0	36 ± 11.1	31 ± 6.8	30 ± 5.6
AST (U/l)	63 ± 9.4	68 ± 23.5	61 ± 8.8	66 ± 7.2
ALT (U/l)	30 ± 7.0	27 ± 4.4	31 ± 9.4	29 ± 4.6
Calcium (mmol/l)	2.64 ± 0.05	2.60 ± 0.12	2.64 ± 0.10	2.62 ± 0.08
Phosphorus (mmol/l)	1.28 ± 0.23	1.42 ± 0.22	1.49 ± 0.27	1.41 ± 0.32

From Moore (2009)
ALT, alanine aminotransferase; AST, aspartate aminotransferase; U, units; * $P < 0.05$; ** $P < 0.01$

Table 109. Selected group mean urinalysis values after 13 weeks of treatment in rats

Parameter (unit)	Group mean value ± standard deviation			
	Dietary concentration (ppm)			
	0	1000	4000	16 000
Males				
pH	8.1 ± 0.88	7.9 ± 0.73	7.7 ± 1.02	6.6 ± 0.50**
Protein (g/l)	0.99 ± 0.29	1.25 ± 0.13	1.29 ± 0.41	1.37 ± 0.50*
Females				
pH	7.8 ± 1.11	7.5 ± 1.17	6.5 ± 1.06*	6.4 ± 0.88**
Protein (g/l)	0.16 ± 0.05	0.13 ± 0.03	0.26 ± 0.22	0.17 ± 0.06

From Moore (2009)
* $P < 0.05$; ** $P < 0.01$

Specifically, there were no treatment-related histopathological alterations in the liver or kidneys of males at 16 000 ppm.

The NOAEL was 4000 ppm (equal to 258 mg/kg bw per day), based on reduced body weight, body weight gain and feed consumption in males at 16 000 ppm (equal to 1038 mg/kg bw per day) (Moore, 2009).

In two independent microbial mutagenicity tests, *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* strain WP2*uvrA* were exposed to DM-PCA (purity 99.71%) in dimethyl sulfoxide (DMSO) at five dose levels up to 5000 µg/plate, with or without exogenous metabolic activation (S9). No cytotoxicity was observed at any dose level in either test. The incidences of revertant colonies at all levels of DM-PCA, 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, DM-PCA did not induce gene mutation in any of the strains employed, either with or without metabolic activation, at concentrations up to 5000 µg/plate (Sarada, 2005a).

In an in vitro chromosomal aberration test, Chinese hamster lung fibroblast cell line CHL/IU was exposed to DM-PCA (purity 99.71%), prepared in DMSO, to evaluate the clastogenic potential

of test material concentrations ranging from 225 to 1800 µg/ml (equivalent to 10 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, 1800 µg/ml was selected as the highest concentration for the short-term treatment –S9 and +S9 assays and continuous treatment 24-hour assay. The incidence of cells with structural aberrations and the incidence of polyploid cells in the groups treated with DM-PCA were comparable to those of the negative control group in all three assays. Both positive controls induced structural chromosomal aberrations at a markedly higher incidence compared with the negative controls. DM-PCA did not induce chromosomal aberrations in cultured mammalian cells under the conditions of this study (Nakajima, 2007).

In a mammalian cell forward gene mutation assay in mouse lymphoma cells (L5178Y tk+/− 3.7.2C), the microtitre plating method was used to evaluate the mutagenic potential of DM-PCA (purity 99.71%), prepared in DMSO solvent. On the basis of the results of a dose-finding study, the gene mutation assay was performed with 11 DM-PCA concentrations of 1.76, 3.52, 7.03, 14.1, 28.1, 56.3, 113, 225, 450, 900 and 1800 µg/ml (equivalent to 10 mmol/l), as required by the test guidelines, in the short-term (3-hour) treatment –S9 and +S9 assays and continuous treatment 24-hour assay −S9. Methyl methanesulfonate and cyclophosphamide positive controls were run concurrently.

Relative total growth was greater than 10% at the high concentration of 1800 µg/ml (equivalent to 10 mmol/l) under all assay conditions. The mutant frequency was not increased at any concentration up to 1800 µg/ml in the short-term treatment –S9 or +S9 assay or the continuous 24-hour treatment assay, as compared with the negative control. Both positive controls induced high incidences of gene mutation, and all criteria for a valid assay were satisfied. It was concluded that DM-PCA did not induce gene mutation in mammalian cultured cells under the conditions of this study (Masumori, 2007).

Metabolite PCA (1-methyl-3-trifluoromethyl-1H-pyrazole-4-carboxylic acid)

In an acute oral toxicity study (acute toxic class method), single oral gavage doses of PCA (purity 99.92%) were administered as an aqueous methylcellulose suspension, at 10 ml/kg bw, to two groups of three food-deprived female Crl:CD Sprague-Dawley (SPF) 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 or clinical signs of an adverse reaction to treatment occurred, and 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 (Oda, 2005b).

In a subchronic (28-day) oral toxicity study in Wistar rats, four groups, each comprising five male and five female rats, were treated orally, by gavage, with 10 ml/kg bw of an aqueous suspension of PCA (purity 99.1%) at a dose level of 0 (vehicle only), 100, 300 or 1000 mg/kg bw per day for 28 days. Dose levels for the study were determined on the basis of a 5-day range-finding study. Clinical signs were recorded daily, and a detailed examination of each animal was performed weekly (weeks 1–3). During acclimatization and in week 4, FOB, quantitative grip strength and motor activity measurements were performed on all animals. Feed consumption and body weights were recorded weekly, and blood samples for haematology and plasma chemistry and urine for urinalysis 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. There were no treatment-related changes evident during the FOB observations or on grip strength and motor activity in week 4. Feed consumption and body weight gain were unaffected by treatment at all dose levels in both sexes. There were no treatment-related effects at any dose level in either sex on the haematological and plasma chemistry profiles. Urinalysis 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 1000 mg/kg bw per day.

The NOAEL was 1000 mg/kg bw per day (limit dose). A LOAEL was not determined (Braun, 2008).

In independent trials of a reverse gene mutation assay, PCA (purity 99.92%), prepared in DMSO, was tested in four strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537) and in *Escherichia coli* strain WP2*uvrA* in a pre-incubation assay at six dose levels up to 5000 µg/plate with or without S9 activation.

In the main assay, six dose levels from 156 µg/plate up to and including 5000 µg/plate with or without S9 were assayed. The incidences of revertant colonies at all concentrations of PCA, in all strains, both with and without S9, were comparable to the corresponding solvent control incidences. The numbers of revertant colonies in all strain-specific positive control groups were clearly increased. The numbers of revertant colonies in the solvent and all positive control groups were within the laboratory historical control ranges.

Under the conditions of this study, PCA did not induce gene mutation in any of the strains employed, either with or without metabolic activation, at concentrations up to 5000 µg/plate (Mochizuki, 2005).

In an in vitro chromosomal aberration test, Chinese hamster lung fibroblast cells (V79) were exposed to PCA (purity 99.86%) prepared in DMSO. Concentrations ranging from 62.5 to 2000 µg/ml (equivalent to 10 mmol/l) with or without S9 were generally tested in three independent experiments (Table 110). Doses for the main assay were determined following a preliminary cytotoxicity test. On the basis of these results, 2000 µg/ml was selected as the high concentration for the 4-, 18- or 28-hour harvests in the absence of S9 and the 4-hour exposure with harvest times of 18 and 28 hours in the presence of S9. Concentrations of 500, 1000 and 2000 µg/ml were scored for chromosomal aberrations under all test conditions, and the pH of the highest concentrations tested (500, 1000 and 2000 µg/ml) was adjusted to physiological values for all experiments.

In all experiments in the absence and presence of S9, no cytotoxicity or test material precipitation was observed up to the highest applied concentration. In all experiments, regardless of exposure or harvest time, the highest applied and evaluated concentration of 2000 µg/ml with or without S9 was not clastogenic. Similarly, in experiments I and II at the 28-hour harvest in the absence and presence of S9, no clastogenicity was observed at the concentrations evaluated.

However, in experiment II (18-hour harvest –S9), significant ($P < 0.05$) increases in the numbers of aberrant cells excluding gaps (7.5% and 6.0%) were observed after treatment with 500 and 1000 µg/ml, respectively (experiment IIA). These findings conflict with data showing that treatment with 2000 µg/ml without S9 did not lead to an increased number of aberrant cells (2.0% aberrant cells excluding gaps). Accordingly, a repeat test (experiment IIB) was conducted to clarify these results. Findings from the repeated experiment IIB indicated that 500 µg/ml without S9 induced 1.5% aberrant cells excluding gaps. Therefore, the former significantly higher incidence of aberrant cells was not reproduced. Additionally, in experiment IIA at the 28-hour harvest, 500 µg/ml without S9 did not increase the number of cells with chromosomal aberrations (1.5% aberrant cells excluding gaps). The

Table 110. Summary of results of the chromosomal aberration study with PCA

Experiment	Harvest interval (h)	Test item concentration (µg/ml)	Polyploid cells (%)	Cell numbers (% of control)	Mitotic indices (% of control)	Aberrant cells (%)		
						Including gaps	Excluding gaps	With exchanges
4 h exposure –S9								
I	18	Solvent control[a]	1.5	100.0	100.0	2.0	1.5	0.0
		Positive control[b#]	1.8	n.t.	94.8	35.0	33.0*	18.0
		500	2.1	n.e.	117.7	1.5	1.0	0.5
		1000	1.7	106.0	120.7	2.0	2.0	1.0
		2000	1.8	79.8	117.2	3.0	2.5	0.0
18 h exposure –S9								
IIA	18	Solvent control[a]	2.9	100.0	100.0	2.5	1.5	0.0
		Positive control[c]	1.8	n.t.	105.2	15.0	14.5*	3.5
		500	2.9	68.1	118.9	8.0	7.5*	1.5
		1000	2.6	76.1	109.9	7.0	6.0*	1.5
		2000	3.1	70.4	99.5	4.5	2.0	0.0
IIB	18	Solvent control[a]	2.7	100.0	100.0	3.0	2.0	0.0
		Positive control[c#]	3.0	n.t.	101.7	40.0	33.0*	8.0
		500	2.4	110.4	90.4	2.5	1.5	0.5
		1000[##]	3.3	87.1	95.7	6.3	5.3*	1.5
		2000	2.6	89.9	80.0	3.0	1.5	0.0
28 h exposure −S9								
IIA	28	Solvent control[a]	2.2	100.0	100.0	2.5	2.5	0.5
		Positive control[c]	1.9	n.t.	68.5	27.5	26.5*	16.0
		500	1.8	78.4	114.8	1.5	1.5	0.0
		1000	1.7	66.1	93.5	1.5	1.5	0.5
		2000[##]	3.0	65.3	114.5	4.5	3.0	1.5
4 h exposure +S9								
I	18	Solvent control[a]	1.5	100.0	100.0	2.5	2.5	0.5
		Positive control[d]	1.5	n.t.	73.0	15.0	14.5*	3.5
		500	1.3	102.8	93.4	2.0	2.0	1.0
		1000	1.7	97.2	95.9	2.5	2.5	0.5
		2000	1.1	98.7	100.0	3.5	3.5	1.0
IIA	28	Solvent control[a]	2.5	100.0	100.0	3.0	2.0	0.0
		Positive control[e]	1.7	n.t.	114.3	13.5	13.0*	5.0
		500	2.5	114.2	118.5	0.5	0.5	0.0
		1000	1.9	96.5	110.3	1.5	1.0	0.0
		2000	1.8	116.3	104.9	2.0	1.5	0.5

From Hoffmann (2008). Data were derived from report table 1, pp. 24 and 25.

n.e., not evaluated because of technical issues; n.t., not tested; [#] evaluation of 50 metaphase plates per culture; [##] evaluation of 200 metaphase plates per culture; * aberration frequency significantly ($P < 0.05$) higher than corresponding control values

[a] DMSO 0.5% (v/v).

[b] Ethyl methanesulfonate 900 µg/ml.

[c] Ethyl methanesulfonate 500 µg/ml.

[d] Cyclophosphamide 1.4 µg/ml.

[e] Cyclophosphamide 2.0 µg/ml.

pulse treatment (4 hours) with 500 µg/ml without metabolic activation in experiment I also did not induce an increased number of aberrations (1.0% aberrant cells excluding gaps).

In experiment IIA at 1000 µg/ml without S9, 6.0% aberrant cells excluding gaps were scored. In the repeated experiment IIB, this observation of an increased number of chromosomal aberrations at a comparable dose was reproduced (5.3% aberrant cells excluding gaps). However, the 28-hour harvest with 1000 µg/ml was negative (1.5% aberrant cells excluding gaps). Also, the pulse treatment in experiment I did not induce higher frequencies of chromosomal aberrations (2.0% aberrant cells excluding gaps) with this concentration.

Similarly, no relevant increase in the frequencies of polyploid metaphases was found after treatment with the test material compared with the frequencies of the controls. The appropriate mutagens induced the expected significant ($P < 0.05$) increases in cells with structural chromosomal aberrations.

In consideration of all of the data and their biological relevance, it was concluded that PCA did not produce a reproducible clastogenic effect at concentrations up to and including 2000 µg/ml. Therefore, PCA is considered to be non-clastogenic in this chromosomal aberration test (Hoffmann, 2008).

In a mammalian cell forward gene mutation assay in mouse lymphoma cells (L5178Y tk+/− 3.7.2C), the microtitre plating method was conducted to evaluate the mutagenic potential of PCA (purity 99.92%) prepared in DMSO. On the basis of the results of a dose-finding study, the gene mutation assay was performed with 10 concentrations of 3.79, 7.58, 15.2, 30.3, 60.7, 121, 243, 485, 971 and 1941 µg/ml (equivalent to 10 mmol/l) in the short-term (3-hour) treatment –S9 and +S9 assays and continuous treatment 24-hour assay. Methyl methanesulfonate and cyclophosphamide positive controls were run concurrently.

No significant pairwise or trend increases in mutant frequency were noted in the PCA-treated groups in the short-term treatment –S9 assay. Therefore, the response of PCA was judged negative in the short-term treatment –S9 assay (Table 111). A significant ($P \leq 0.05$) dose-related trend in mutant frequency was observed in the PCA-treated groups in the short-term +S9 assay, but significant pairwise increases in mutant frequency were not observed, and none of the mutant frequency values up to 10 mmol/l exceeded twice the contemporary negative control value or the global evaluation factor (126×10^{-6} + the solvent control mutant frequency [189×10^{-6}] = 315×10^{-6}) recommended by the International Workshop on Genotoxicity Testing for the mouse lymphoma thymidine kinase mutation assay (Moore et al., 2006).

In the continuous 24-hour treatment assay, a significant ($P \leq 0.05$) increase in mutant frequency values and a significant ($P \leq 0.05$) trend were noted in the PCA-treated groups. The highest mutant frequency value was 2.23-fold the contemporary negative control value and also greater than the mean historical control value + 3 standard deviations. The mutant frequency at 243 and 485 µg/ml also exceeded the global evaluation factor (126×10^{-6} + the solvent control mutant freqency [195×10^{-6}] = 321×10^{-6}) recommended above by Moore et al. (2006). These results suggested a weak positive mutagenic response for PCA. However, the highest mutant frequency value occurred at 485 µg/ml, and two higher concentrations (971 and 1941 µg/ml), with acceptable cytotoxicity (≥ 10% cell survival), had mutant frequency values only slightly higher than the negative control value (1.32-fold and 1.16-fold, respectively), with relative total growth for both levels at approximately 70%. Therefore, based on considerations of biological plausibility, the response to PCA was considered to be equivocal.

No relevant increase in the ratio of small to large colonies, which is related to induction of chromosomal aberration, was observed compared with the negative control group in any assays. Both positive controls induced gene mutation at a high incidence, and all criteria for a valid assay were satisfied.

Table 111. Mouse lymphoma gene mutation assay with PCA: continuous treatment for 24 hours

Substance	Concentration (µg/ml)	Exposure time (h)	PE0 (%)	RS (%)	RSG (%)	RTG (%)	PE2 (%)	Mutant frequency (× 10^{-6})			Inductivity	% SC
								Large	Small	Total		
DMSO[a]	0	24	135.3	100.0	100.0	100.0	85.7	124.0	62.5	194.8[†]	1.00	32.1
PCA	3.79	24	125.0	92.4	93.6	114.3	104.6	121.2	69.5	212.8	1.09	32.7
	7.58	24	163.7	121.0	94.6	115.6	104.6	118.0	81.2	208.9	1.07	38.9
	15.2	24	116.0	85.8	97.2	111.2	98.0	122.5	80.4	210.8	1.08	38.1
	30.3	24	125.0	92.4	107.8	123.3	98.0	139.7	74.2	223.0	1.14	33.3
	60.7	24	163.7	121.0	91.4	87.1	81.6	151.2	67.4	220.3	1.13	30.6
	121	24	155.3	114.8	80.6	89.3	95.0	133.5	89.4	225.9	1.16	39.6
	243	24	135.4	100.1	81.5	77.7	81.6	189.1	127.2	346.7*	1.78	36.7
	485	24	116.0	85.8	107.3	88.5	70.6	249.3	147.0	433.9[#]*	2.23	33.9
	971	24	104.6	77.3	69.7	77.2	95.0	119.5	109.3	256.2	1.32	42.7
	1941	24	81.6	60.4	70.2	64.9	79.3	143.2	69.4	226.8	1.16	30.6
MMS[b]	5.00	24	112.0	82.8	117.2	83.8	61.3	383.4	540.3	1300.1	6.67	41.6

From Tanaka (2009a)

DMSO, dimethyl sulfoxide; MMS, methyl methanesulfonate; PE0, plating efficiency on day 0; PE2, plating efficiency on day 2; RS, relative survival on day 0; RSG, relative suspension growth; RTG, relative total growth; % SC, ratio of small colony [(small mutant frequency/total mutant frequency) ×100]; SD, standard deviation

[†] significant difference by trend test, $P \leq 0.05$; [#] significant difference from control by modified Dunnett test, $P \leq 0.05$; greater than the mean historical control value + 3 SD (mean ± SD: 146.6 ± 36.8 × 10^{-6});

* mutant frequency at 243 and 485 µg/ml exceeded the global evaluation factor (126 × 10^{-6} + the solvent control mutant frequency [195 × 10^{-6}] = 321 × 10^{-6})

[a] Negative control (10 µl/ml).

[b] Positive control.

Based on the results of the continuous 24-hour exposure treatment regimen, PCA at 243 and 485 µg/ml induced mutant frequencies that were judged to be weakly positive. However, as higher concentrations did not produce positive responses, the weakly positive result is of questionable biological plausibility. Therefore, it was concluded that PCA induced an equivocal mutagenic response in mammalian cultured cells under the conditions of this study (Tanaka, 2009a).

In an in vivo bone marrow micronucleus assay, PCA (purity 99.1%) prepared in suspensions in methylcellulose was administered via gavage to BDF1 mice (six males per dose) once daily for 2 consecutive days at a dose level of 0 (vehicle), 500, 1000 or 2000 mg/kg bw per day.

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. In contrast, the mean frequency of micronucleated polychromatic erythrocytes in the mitomycin C–treated positive control group was significantly ($P \leq 0.25$) higher than that of the control group by a factor greater than 7-fold. There was no significant difference in the proportion of polychromatic erythrocytes relative to total erythrocytes between any of the PCA-treated groups and the negative control group. Mitomycin C also did not induce a significant decrease in the proportion of polychromatic erythrocytes. 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.

Toxicokinetic measurements were conducted to confirm systemic exposure to PCA in a group of four male mice treated once orally, by gavage, at 500 mg/kg bw. PCA was analysed in whole blood samples withdrawn 1 hour after treatment. The mean concentration of PCA in whole blood was 124.5 µg/ml 1 hour after dosing. Individual animal values were within the range 68.16–205.0 µg/ml. Therefore, it was inferred that PCA was absorbed systemically in male mice at all dose levels employed and that the treatment regime maximized the likelihood of exposure of the target cell population in bone marrow.

It was concluded that PCA 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 (Tanaka, 2009b).

Metabolite PAM (1-methyl-3-trifluoromethyl-1H-pyrazole-4-carboxamide)

In an acute oral toxicity study (acute toxic class method), single oral gavage doses of PAM (purity 99.97%) were administered as an aqueous methylcellulose suspension, at 10 ml/kg bw, to three groups of three feed-deprived female Crl:CD Sprague-Dawley (SPF) rats. An initial group was dosed at 2000 mg/kg bw, and two subsequent groups were dosed at 300 mg/kg bw. All surviving animals were observed for 14 days post-treatment and then subjected to necropsy and postmortem examination.

All three animals treated at 2000 mg/kg bw died within 6 hours of dosing, but no deaths occurred in either of the groups treated at 300 mg/kg bw. At 2000 mg/kg bw, death was preceded by decreased activity, tremor and clonic convulsion, but there were no clinical signs of an adverse reaction to treatment at 300 mg/kg bw. There were no macroscopic findings at necropsy in any animal. No overt body weight effects were evident in surviving animals, all of which 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 300 mg/kg bw and less than 2000 mg/kg bw. As per Organisation for Economic Co-operation and Development (OECD) Test Guideline 423 (Annex 2), the LD_{50} cut-off value for this outcome is 500 mg/kg bw (Oda, 2005c).

In a second acute oral toxicity study (acute toxic class method), single oral gavage doses of PAM (purity 100.00%) were administered as an aqueous methylcellulose suspension at 10 ml/kg bw

to three groups of three feed-deprived male Crl:CD Sprague-Dawley (SPF) rats. An initial group was dosed at 300 mg/kg bw. This was followed by a second group at the same dose and a third group at 2000 mg/kg bw. All surviving animals were observed for 14 days post-treatment and then subjected to necropsy and postmortem examination.

No deaths occurred in either of the groups treated at 300 mg/kg bw, but all three animals treated at 2000 mg/kg bw died within 4–6 hours of dosing. There were no clinical signs of an adverse reaction to treatment at 300 mg/kg bw, but at 2000 mg/kg bw, death was preceded by decreased activity, tremor and clonic convulsion. There were no macroscopic findings at necropsy in any animal. No overt body weight effects were evident in surviving animals, all of which 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 300 mg/kg bw and less than 2000 mg/kg bw. As per OECD Test Guideline 423 (Annex 2), the LD_{50} cut-off value for this outcome is 500 mg/kg bw (Oda, 2009).

In two independent trials of a reverse gene mutation assay, PAM (purity 99.97%), prepared in DMSO solvent, was tested in four strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537) and in *Escherichia coli* WP2*uvrA* in a pre-incubation reverse mutation assay at five concentrations up to 5000 µg/plate with and without exogenous metabolic activation. The incidences of revertant colonies at all concentrations of PAM, in all strains, both with and without S9, were comparable to the corresponding solvent control incidences. The numbers of revertant colonies in all strain-specific positive control groups were clearly increased. The numbers of revertant colonies in the solvent and all positive control groups were within the laboratory historical control ranges. Under the conditions of this study, PAM did not induce gene mutation in any of the strains employed, either with or without metabolic activation, at concentrations up to 5000 µg/plate (limit concentration) (Sarada, 2005b).

In an in vitro chromosomal aberration test, Chinese hamster lung fibroblast cell line CHL/IU was exposed to PAM (purity 99.97%), prepared in DMSO, to evaluate the clastogenic potential of test material concentrations ranging from 483 to 1931 µg/ml with or without S9. On the basis of the results of a preliminary cytotoxicity assessment of cell growth inhibition, a chromosomal aberration test was conducted up to the concentration of 1931 µg/ml (equivalent to 10 mmol/l) in the short-term treatment –S9 and +S9 assays and continuous treatment 24-hour assay. The incidences of cells with structural aberrations and the incidences of polyploid cells in the groups treated with PAM were comparable to those of the negative control group in the short-term treatment –S9 and +S9 assays. However, in the continuous treatment 24-hour assay without S9, the percentage of cells with structural chromosomal aberrations was 16.0% at 1931 µg/ml.

To confirm the reproducibility or the concentration dependency in the continuous treatment 24-hour assay, the confirmation study was performed. The reproducibility of the relative cell growth was not confirmed from the results of the chromosomal aberration test and confirmation study. Therefore, a reconfirmation study was conducted. In the reconfirmation study, microscopic examinations of the mitotic cells were performed at the four concentrations of 989, 1236, 1545 and 1931 µg/ml. In this study, the percentages of cells with structural chromosomal aberrations were 7.5% (989 µg/ml), 13.0% (1236 µg/ml), 17.5% (1545 µg/ml) and 14.5% (1931 µg/ml), and there was clear concentration dependency. The most frequently observed type of chromosome damage was chromatid breaks and exchanges. The percentage of polyploid cells in the test groups was comparable to that in the negative control group in the short-term and continuous treatment 24-hour assays. Both positive controls induced structural chromosomal aberrations at a markedly higher incidence compared with the negative controls.

It was concluded that PAM induced chromosomal aberrations in cultured mammalian cells in the absence of S9 following exposure for 24 hours (Nakajima, 2009).

In a mammalian cell forward gene mutation assay in mouse lymphoma cells (L5178Y tk+/– 3.7.2C) using the microtitre plating method, the mutagenic potential of PAM (purity 99.97%), prepared in DMSO, was evaluated. On the basis of the results of the dose-finding study, the gene mutation assay was performed with 10 concentrations of 3.77, 7.54, 15.1, 30.2, 60.3, 121, 241, 483, 966 and 1931 µg/ml (equivalent to 10 mmol/l), as required by the test guidelines, in the short-term (3-hour) treatment –S9 and +S9 assays and the continuous 24-hour non-activated treatment assay.

The mutant frequency was not increased at any concentration up to 1931 µg/ml (equivalent to 10 mmol/l) in the short-term treatment –S9 or +S9 assay, compared with the negative control. However, a significant and dose-dependent increase in the mutant frequency was noted in the PAM-treated groups in the continuous treatment 24-hour non-activated portion of the assay (Table 112). In addition, the mutant frequency value at the highest relevant concentration of 483 µg/ml was increased to more than 3 standard deviations + the mean historical control data (mutant frequency [$\times 10^{-6}$] mean ± standard deviation: 146.6 ± 36.8) and over twice the negative control value (131.0 $\times 10^{-6}$) and exceeded the global evaluation factor (126 $\times 10^{-6}$ + the solvent control mutant frequency [131 $\times 10^{-6}$] = 257 $\times 10^{-6}$) recommended by the International Workshop on Genotoxicity Testing for the mouse lymphoma thymidine kinase mutation assay (Moore et al., 2006). Based on the number of mutant colonies, rather than the mutant frequency, the increase was only in the number of small colonies, an indication of chromosomal aberration rather than gene mutation. The continuous exposure for 24 hours to concentrations of 966 and 1931 µg/ml also produced high mutant frequency values, but the relative total growth was 5.1% and 0.3%, respectively. Nevertheless, the response showed a significant relationship with concentration and fulfilled the consensus criteria for a positive effect in this test system, based on international agreements (Moore et al., 2006). The ratio of small to large colonies was also increased at the highest concentration, but it was considered secondary to cytotoxicity, because the relative total growth was less than 1%. Both positive controls induced marked increases in the mutant frequency.

It was concluded that continuous non-activated exposure to PAM for 24 hours induces gene mutations in mammalian cultured cells under the conditions of this study (Masumori, 2009a).

In an in vivo micronucleus assay, PAM (purity 100%) prepared as suspensions in 0.5% methylcellulose was administered via gavage to B6D2F1(BDF1)[SPF] mice (six of each sex per dose). PAM was administered once daily for 2 consecutive days at a dose level of 0 (vehicle), 125, 250 or 500 mg/kg bw per day in males and 0 (vehicle), 500, 1000 or 2000 mg/kg bw per day in females. As a result of the deaths of four out of six females at 2000 mg/kg bw per day, an additional study was conducted in females at 0, 250, 500 and 1000 mg/kg bw per day.

There were no deaths, adverse clinical signs or apparent effects on body weight gain in main study males and the additional study in females. The group mean frequencies of micronucleated polychromatic erythrocytes in the treated groups of both sexes were similar to the vehicle control frequencies, and none was significantly different from the control frequencies. In contrast, the mean frequencies of micronucleated polychromatic erythrocytes in the mitomycin C–treated positive control groups of both sexes were significantly higher ($P \leq 0.025$) than the control group values by a factor of at least 4.5-fold.

There was no significant difference in the proportion of polychromatic erythrocytes relative to total erythrocytes between any of the PAM-treated groups and the negative control group. The proportion of polychromatic erythrocytes in mitomycin C–treated mice was significantly decreased only for the males.

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.

Table 112. Mouse lymphoma gene mutation assay with PAM: continuous treatment for 24 hours without S9

Substance	Concentration (µg/ml)	Exposure time (h)	PE0 (%)	RS (%)	RSG (%)	RTG (%)	PE2 (%)	Mutant frequency (× 10^{-6})			Fold increase	% SC
								Large	Small	Total		
DMSO[a]	0	24	109.5	100.0	100.0	100.0	91.7	45.1	82.6	131.0[†]	1.00	63.1
PAM	3.77	24	130.0	118.6	117.5	98.7	77.0	67.7	102.3	182.3	1.39	56.1
	7.54	24	141.4	129.0	107.0	107.5	92.1	53.5	109.3	175.4	1.34	62.3
	15.1	24	141.4	129.0	111.3	140.8	116.0	44.9	89.5	142.3	1.09	62.9
	30.2	24	135.4	123.6	107.7	87.9	74.8	62.0	117.7	192.3	1.47	61.2
	60.3	24	84.1	76.8	116.2	124.2	98.0	62.1	112.5	187.2	1.43	60.1
	121	24	116.0	105.9	119.1	123.3	95.0	70.3	123.0	189.3	1.45	65.0
	241	24	112.0	102.2	94.0	79.0	77.0	79.0	151.7	243.3	1.86	62.3
	483	24	36.0	32.8	43.0	35.1	74.8	81.3	173.9	265.8*	2.03	65.4
	966	24	15.4	14.1	9.3	5.1	50.2	109.6	219.7	350.8*	2.68	62.6
	1931	24	4.0	3.7	4.0	0.3	7.6	103.5	647.1	761.4*	5.81	85.0
MMS[b]	5.00	24	57.9	52.9	101.5	71.8	64.9	288.8	834.8	1290.2*	9.85	64.7

From Masumori (2009a). Data were derived from report table 5, p. 42.

DMSO, dimethyl sulfoxide; MMS, methyl methanesulfonate; PE0, plating efficiency on day 0; PE2, plating efficiency on day 2; RS, relative survival on day 0; RSG, relative suspension growth; RTG, relative total growth; % SC, ratio of small colony [(small mutant frequency /total mutant frequency) × 100]

[†] significant difference by trend test, $P \leq 0.05$; * significantly increased compared with control by modified Dunnett's test, $P \leq 0.05$; increased up to 3 standard deviations + the mean historical control data (mutant frequency × 10^{-6}, mean ± standard deviation: 146.6 ± 36.8) and exceeded twice the negative control value; exceeded the global evaluation factor (126 × 10^{-6} + the solvent control mutant frequency [131 × 10^{-6}] = 257 × 10^{-6}) recommended by the International Workshop on Genotoxicity Testing for the mouse lymphoma thymidine kinase mutation assay (Moore et al., 2006).

[a] Negative control (10 µl/ml).

[b] Positive control.

Toxicokinetic measurements were conducted to confirm the systemic exposure to PAM in a group of four male mice treated once orally, by gavage, at 125 mg/kg bw and two groups of four female mice treated once orally, by gavage, at 250 and 500 mg/kg bw. PAM was analysed in whole blood samples withdrawn 1 hour after treatment. The mean concentration of PAM in male whole blood was 130.8 µg/ml at 1 hour after dosing of 125 mg/kg bw, and individual animal values were within the range 120.8–135.9 µg/ml. In females, the mean concentrations of PAM in whole blood were 182.7 µg/ml (individual value: 162.6–194.4 µg/ml) and 368.1 µg/ml (individual value: 288.7–422.5 µg/ml) at 1 hour after dosing of 250 and 500 mg/kg bw, respectively. Therefore, it was inferred that PCA was absorbed systemically in male mice at all dose levels employed and that the treatment regimen maximized the likelihood of exposure of the target cell population in bone marrow.

It was concluded that PAM did not induce the formation of micronucleated polychromatic erythrocytes in male or female mouse bone marrow cells at levels up to the maximum tolerated dose under the conditions employed for this study (Masumori, 2009b).

Metabolite 753-A-OH (N-[2-(3-hydroxy-1,3-dimethyl-butyl) thiophen-3-yl]-1-methyl-3-trifluoromethyl-1H-pyrazole-4-carboxamide)

In an acute oral toxicity study (acute toxic class method), single oral gavage doses of 753-A-OH (purity 98.91%) were administered as an aqueous methylcellulose suspension, at 10 ml/kg bw, to two groups of three feed-deprived female Crl:CD Sprague-Dawley (SPF) 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 or clinical signs of an adverse reaction to treatment occurred, and 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 (Oda, 2005d).

In independent trials of a reverse gene mutation assay, 753-A-OH (purity 98.1%) prepared in DMSO was tested in four strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537) and in *Escherichia coli* strain WP2*uvrA* in a pre-incubation assay at five or six concentrations ranging from 39 to 5000 µg/plate with or without S9 activation. The highest dose level used for the two main assays was set at 1250 µg/plate for the *S. typhimurium* TA strains, and a total of six dose levels by five stepwise dilutions using a common ratio of 2 were prepared. A maximum concentration of 5000 µg/plate and a total of five dose levels by four stepwise dilutions using a common ratio of 2 were provided for the *E. coli* strain.

Growth inhibition due to the test substance was observed in *S. typhimurium* TA strains with and without metabolic activation at and above 1250 µg/plate. Precipitation of the test substance on the plate was observed with and without metabolic activation at and above 1250 µg/plate. However, the mean numbers of revertant colonies for all strains were not appreciably increased by treatment with the test substance at all concentrations, with and without S9. The numbers of revertant colonies in all strain-specific positive control groups were clearly increased and were within the laboratory historical control ranges.

Under the conditions of this study, 753-A-OH did not induce gene mutation, either with or without metabolic activation, in any of the *S. typhimurium* TA strains up to concentrations that inhibited cell growth or in the *E. coli* strain up to a concentration of 5000 µg/plate (Sarada, 2006).

In an in vitro chromosomal aberration test, Chinese hamster lung (CHL) fibroblast cells were exposed to 753-A-OH (purity 98.9%), prepared in DMSO, in two independent experiments. Concentrations ranging from 6.25 to 1000 µg/ml with or without S9 were used to evaluate the clastogenic potential of the test material. On the basis of the results of the cytotoxicity range-finding experiment, main experiments were conducted up to maximum concentrations of 500 or 1000 µg/ml using

3-hour exposures with and without metabolic activation and 20- and 3-hour exposures without and with metabolic activation, respectively. Metaphases were examined for cells treated with 50–200 μg/ml; higher doses were not evaluated owing to precipitation of the test material.

The incidences of cells with structural aberrations and numerical aberrations in all groups treated with 753-A-OH were comparable to those of the negative control in all assays. Both positive controls induced significant increases in the proportion of cells with structural chromosomal aberrations compared with the negative controls, and all assay validity criteria were achieved.

It was concluded that 753-A-OH did not induce chromosomal aberrations in cultured mammalian cells under the conditions in this study at concentrations up to and including the limit of solubility and/or cytotoxicity (Lloyd, 2009a).

In a mammalian cell forward gene mutation assay, mouse lymphoma cells (L5178Y tk+/−) were assayed using the microtitre plating method to evaluate the mutagenic potential of 753-A-OH (purity 98.91%) prepared in DMSO. On the basis of the results of precipitation in the dose range–finding study, the highest concentration of the main study was selected. In experiment 1, 10 concentrations, ranging from 25 to 500 μg/ml, were tested for 3-hour treatments with and without S9. Two days after treatment, the highest concentrations selected to determine viability and trifluorothymidine resistance were limited by the appearance of post-treatment precipitate from 150 μg/ml without S9 and 250 μg/ml with S9, which gave 61% and 91% relative total growth, respectively.

In experiment 2, 11 concentrations, ranging from 10 to 400 μg/ml, were tested for 24-hour treatment without S9 and for 3-hour treatment with S9. Two days after treatment, the highest concentrations selected to determine viability and trifluorothymidine resistance were 80 μg/ml for 24-hour treatment without S9 (limited by cytotoxicity at 100–150 μg/ml) and 250 μg/ml for 3-hour treatment with S9 (limited by the appearance of post-treatment precipitate), which gave 15% and 127% relative total growth, respectively.

The mutation frequencies of 753-A-OH concentrations plated, for all treatment regimens, were less than the sum of the mean control mutant frequency plus the global evaluation factor: (78 + 126 mutants per 10^6 viable cells = 204 mutants per 10^6 viable cells [3 hours –S9]; 83 + 126 mutants per 10^6 viable cells = 209 mutants per 10^6 viable cells [3 hours +S9]; and 102 + 126 mutants per 10^6 viable cells = 228 mutants per 10^6 viable cells [24 hours –S9]), indicating a negative result. No significant linear trends were observed.

Mutant frequencies in negative control cultures fell within acceptable ranges, and clear increases in mutation were induced by the positive controls, methyl methanesulfonate (without S9) and benzo[*a*]pyrene (with S9).

It was concluded that 753-A-OH did not induce gene mutations in mammalian cultured cells under the conditions that included treatments up to precipitating and/or cytotoxic concentrations, with and without metabolic activation (Stone, 2009).

Metabolite 753-T-DO (N-[5-hydroxy-5-(1,3-dimethylbutyl)-2-oxo-2,5-dihydrothiophen-4-yl]-1-methyl-3-trifluoromethyl-1H-pyrazole-4-carboxamide)

In independent trials of a reverse gene mutation assay, 753-T-DO prepared in DMSO solvent was tested in four strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537) and in *Escherichia coli* strain WP2*uvrA* in plate incorporation (trial 1) and pre-incubation (trial 2 with S9 mix only) reverse mutation assays at six concentrations up to 1000 μg/plate except for strain WP2*uvrA*, which was tested at six or seven dose levels up to 5000 μg/plate.

The numbers of revertant colonies in all strain-specific positive control groups were clearly increased. The numbers of revertant colonies in the solvent and all positive control groups were within the respective laboratory historical control ranges.

Under the conditions of this study, 753-T-DO did not induce gene mutation in any of the strains employed, either with or without metabolic activation (Williams, 2009).

In an in vitro chromosomal aberration test, Chinese hamster lung (CHL) fibroblast cells were exposed to 753-T-DO (purity 99.84%) prepared in DMSO in two independent experiments. On the basis of the results of the cytotoxicity range-finding experiment, main experiments were conducted at up to maximum concentrations of 40–120 µg/ml, using 3-hour exposures with and without metabolic activation and 20-hour and 3-hour exposures without and with metabolic activation, respectively.

The incidences of cells with structural aberrations and numerical aberrations in the groups treated with 753-T-DO were very similar to those of the negative control group in all assays, with one exception. The number of aberrant cells (excluding gaps) in one culture following treatment at the highest concentration analysed in the presence of S9 in experiment 2 (75 µg/ml) was 4.5% (Table 113), marginally exceeding the normal range (0–4%), but the aberration frequencies (excluding gaps) in the replicate culture at this concentration and in all other cultures analysed in the absence and presence of S9 in both independent experiments were within the normal ranges. Furthermore, there were no increases in aberration frequency (excluding gaps) in any culture analysed up to a maximum of 90 µg/ml in the presence of S9 in experiment 1. Therefore, the single, isolated observation in experiment 2 was not reproduced within or between experiments and was considered to be of no biological relevance.

The incidences of cells with numerical aberrations in the groups treated with 753-T-DO were comparable to those of the negative control group in all assays.

Both positive controls induced statistically significant increases in the proportion of cells with structural chromosomal aberrations compared with the negative controls, and all assay validity criteria were achieved.

It was concluded that 753-T-DO did not induce chromosomal aberrations in cultured mammalian cells under the conditions of this study at concentrations up to and including those producing in excess of 50% cytotoxicity, both with and without metabolic activation (Lloyd, 2009b).

In a mammalian cell forward gene mutation assay, mouse lymphoma cells (L5178Y tk+/−3.7.2C) were assayed using the microtitre plating method to evaluate the mutagenic potential of 753-T-DO (purity 98.84%) prepared in DMSO. On the basis of extreme or complete cytotoxicity in the dose range–finding study, the highest concentrations to provide greater than 10% relative total growth were 31.25 µg/ml (3 hours –S9), 62.5 µg/ml (3 hours +S9) and 31.25 µg/ml (24-hour treatment), which gave 117%, 60% and 11% relative total growth, respectively. For experiment 1, therefore, 10 concentrations, ranging from 10 to 75 µg/ml in the 3-hour treatment without S9, from 10 to 120 µg/ml in the 3-hour treatment with S9 and from 5 to 50 µg/ml in the 24-hour treatment, were tested. Two days after treatment, the highest concentrations selected to determine viability and trifluorothymidine resistance were 70 µg/ml (3 hours −S9), 90 µg/ml (3 hours +S9) and 35 µg/ml (24 hours), which gave 23%, 12% and 13% relative total growth, respectively. In experiment 2, 10 concentrations, ranging from 10 to 120 µg/ml, were tested in the 3-hour treatment with S9. Two days after treatment, the highest concentration selected to determine viability and trifluorothymidine resistance was 100 µg/ml, which gave 19% relative total growth.

The mutation frequencies of 753-T-DO for all concentrations in all treatment regimens were less than the sum of the mean control mutant frequency plus the global evaluation factor (126 mutants per 10^6 viable cells) (Moore et al., 2006) for the appropriate treatment group solvent control values, indicating a negative result. A significant linear trend was observed in experiment 2 (3 hours +S9), but not in experiment 1. However, in the absence of any marked increases in the mutant frequency in

Table 113. Chromosomal aberration test in Chinese hamster lung cells treated with 753-T-DO: short-term treatment with S9; experiment 2

Substance	Concentration (µg/ml)	Exposure time (h)	Cytotoxicity based on PD (%)	No. of cells analysed	No. of cells with structural aberrations						No. of cells with aberrations except gaps (%)	No. of cells analysed for numerical aberrations	No. of numerical aberrations			% of total with numerical aberrations
					gap	csd	cse	ctd	cte	oth			H	E	P	
DMSO[a]	0	3	—	200	0	0	0	5	0	0	5 (2.5)	202	0	0	2	2 (1.0)
753-T-DO	20	3	60	200	1	0	0	0	0	0	0 (0.0)	202	0	0	2	2 (1.0)
	40	3	22	200	0	0	0	1	0	0	1 (0.5)	201	0	0	1	1 (0.5)
	70	3	47	200	0	0	0	0	0	0	0 (0.0)	202	0	0	2	2 (1.0)
	75[b]	3	56	200	0	2	0	7	1	0	9 (4.5)	201	0	0	1	1 (0.5)
CPA[c]	12.5	3	—	56	0	0	0	29	38	6	40* (71.43)	57	0	0	1	1 (1.8)

From Lloyd (2009b). Data were derived from report tables 16 and 20, pp. 42 and 47.

CPA, cyclophosphamide; csd, chromosome deletions; cse, chromosome exchange; ctd, chromatid deletions; cte, chromatid exchange; DMSO, dimethyl sulfoxide; E, endoreduplicated; H, hyperdiploid (28–37 chromosomes): oth, others; P, polyploid (greater than 37 chromosomes); PD, population doubling; * $P \leq 0.001$

[a] Negative control.

[b] Highest concentration tested; higher levels (80, 85, 90, 95, 100 and 120 µg/ml −S9) induced a ≥ 50% decrease in population doubling.

[c] Positive control.

treated cultures in this experiment and the lack of reproducibility of this effect between experiments, this isolated observation was not considered to be biologically relevant.

Mutant frequencies in negative control cultures fell within acceptable ranges, and clear increases in mutation were induced by the positive control substances, methyl methanesulfonate (without S9) and benzo[*a*]pyrene (with S9).

It was concluded that 753-T-DO did not induce gene mutation in mammalian cultured cells under conditions that included treatments up to cytotoxic concentrations in two independent experiments, with and without metabolic activation (Lloyd, 2009c).

3. Observations in humans

Medical data on penthiopyrad 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 penthiopyrad.

Comments

Biochemical aspects

The absorption, distribution, metabolism and excretion of penthiopyrad were investigated in rats. ^{14}C-labelled penthiopyrad was rapidly and extensively absorbed from the gastrointestinal tract of rats following oral dosing. The extent of absorption was approximately 80–90% of the administered dose, independent of dose and sex. Maximum concentrations of radioactivity in plasma were observed within 0.5 hour of dosing for the low-dose group (10 mg/kg bw) and within 1.3 hours for the high-dose group (100 mg/kg bw). Maximum tissue levels occurred within 1 hour post-dosing, with the highest concentrations of radioactivity found in liver, fat, lymph nodes and kidneys of rats. Very little penthiopyrad was retained in the tissues. 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 excreted by all routes 24 hours after dosing (74.8–85.0%).

Extensive metabolism occurred at numerous positions within the molecule, including thienyl ring oxidation and conjugation with glutathione, thienyl ring opening, *N*-demethylation and alkyl side-chain hydroxylation, followed by oxidation to carboxylic acids and glucuronidation. The most abundant metabolite in both urine and faeces was formed as the result of *N*-demethylation and oxidation of the methyl moiety of the alkyl side-chain. The most abundant metabolites found in bile were formed as a result of thienyl ring oxidation to 753-F-DO, followed by its conjugation with glutathione and the catabolism of this product. Other significant metabolites in bile were glucuronic acid conjugates of the intermediate demethylated and hydroxylated metabolites. Four metabolites containing the pyrazole moiety following cleavage from the thienyl moiety were excreted in both urine and faeces. The two acids, PCA and DM-PCA, are likely formed by amide hydrolysis from PAM and DM-PAM. PAM and subsequent metabolites account for less than 1% of the administered dose. The thienyl ring appears to be completely degraded.

Toxicological data

The LD_{50} in rats treated orally and dermally with penthiopyrad was greater than 2000 mg/kg bw. The LC_{50} in rats treated by inhalation was greater than 5.7 mg/l of air. Penthiopyrad was not irritating to the skin of rabbits, was minimally irritating to the eyes of rabbits and was not sensitizing under the conditions of the maximization test in guinea-pigs.

Following repeated dietary dosing, the liver was the main target organ in mice, rats and dogs. In several studies, increased liver weight, liver enlargement and centrilobular hepatocellular hypertrophy were observed, as well as indications of hepatotoxicity in the form of alterations in clinical chemistry (elevated serum levels of liver enzymes, cholesterol, triglycerides and protein). The pattern of liver effects changed with dose, but not with duration of dosing. Haematological changes (decreases in red blood cells, haemoglobin and haematocrit) were observed in mice, rats and dogs at doses higher than those causing liver toxicity. The thyroid was also a target organ in mice, rats and dogs, with effects observed only at the highest doses tested. In mice, thyroid follicular cell hypertrophy was observed in both sexes at 1000 mg/kg bw per day, whereas in dogs, increased thyroid weights were observed in females of the 90-day study at 864 mg/kg bw per day. In longer-term studies in rats, thyroid follicular cell hypertrophy was observed in the 1-year and multigeneration reproduction studies at the highest doses tested. Adrenal cortical hypertrophy was found in both the 90-day and 1-year dog studies at the highest doses tested. Adrenal effects were not observed in mice and were found in rats only with longer-term dosing (i.e. the reproductive toxicity study and long-term study beginning at 6 months).

The NOAEL in the 90-day rat study was 40 mg/kg bw per day, based on liver effects (increased serum levels of phospholipids and GGT, absolute and relative liver weights and incidences of centrilobular hepatocellular hypertrophy, Kupffer cell proliferation and hepatocellular degeneration). The NOAEL in the 90-day mouse study was 100 mg/kg bw per day, and the overall NOAEL in the dog studies was 76.7 mg/kg bw per day, in both cases based on liver effects.

In the 18-month carcinogenicity study in mice, the NOAEL was 60 mg/kg bw per day, based on effects in the liver and thyroid at the LOAEL of 200 mg/kg bw per day. There was a marginal increase in hepatocellular adenomas and carcinomas at the highest dose tested in comparison with concurrent controls; however, the incidences were similar to historical control values, and no other histopathology of the liver was observed. The concurrent control value for adenomas was lower than the historical control range. The Meeting concluded that penthiopyrad was not carcinogenic in mice.

In the 2-year rat study, the NOAEL was 27 mg/kg bw per day, based on reduced body weight gain in females and hepatic periportal fatty degeneration in males at 83 mg/kg bw per day. Effects on the kidneys (various elements of chronic progressive nephropathy, including interstitial fibrosis and renal glomerulosclerosis) were observed in male rats of all groups, including controls. The incidence, but not the severity, of this rat-specific condition was increased to similar extents in all treatment groups. The incidence of thyroid follicular cell adenomas in males was increased at the highest dose tested compared with controls (3/50, 1/50, 6/48, 2/49 and 9/49, respectively); this incidence also slightly exceeded the historical control range. There was no increase in follicular cell carcinomas. No other histopathology of the thyroid was observed in this study; however, follicular cell hypertrophy was observed at higher doses in the 1-year and multigeneration reproduction studies in rats. The Meeting concluded that high doses of penthiopyrad caused follicular cell adenomas in the thyroid.

Hepatocellular adenomas and carcinomas and follicular cell adenomas of the thyroid are common in male mice and rats, respectively. Special studies were conducted to examine liver and thyroid effects in the mouse and rat. These studies showed that penthiopyrad increased microsomal protein and cytochrome P450 activity in the liver of both mice and rats. Changes in thyroid hormones and UDPGT activity were not concordant with the dose–response relationship for the tumours.

Penthiopyrad was adequately tested for genotoxicity in vitro and in vivo in a range of assays. Negative results were observed in all genotoxicity studies.

The Meeting concluded that penthiopyrad was unlikely to be genotoxic.

The Meeting concluded that penthiopyrad is unlikely to pose a carcinogenic risk to humans at anticipated dietary residue levels, as it was not carcinogenic in the mouse and as thyroid follicular cell adenomas in male rats are common, their incidence is only slightly increased and, in the absence of genotoxic potential, the end-point would be anticipated to exhibit a threshold.

No effects on reproduction were noted in a multigeneration reproduction study in the rat. However, there was a decrease in body weight of the offspring during early lactation in both generations at 5000 ppm (equal to 278 mg/kg bw per day), the highest dose tested. Also at this dose, there was a slight, but statistically significant, delay in time to preputial separation. Furthermore, at this dose, there were decreases in thymus and spleen weights, with no histopathological correlates. Effects were observed in parental animals at the intermediate and high doses and included decreased body weight and body weight gain and increased adrenal weight with adrenal cortical hypertrophy. At the high dose only, effects on the thyroid were also observed, comprising increased thyroid weight and follicular cell hypertrophy. The NOAEL for parental toxicity was 200 ppm (equal to 11 mg/kg bw per day), based on decreased body weight gain and effects on the adrenals, whereas the NOAEL for offspring toxicity was 1000 ppm (equal to 54 mg/kg bw per day), based on reduced body weight and body weight gain, delay in preputial separation and a statistically significant decrease in absolute thymus weight at 5000 ppm (equal to 278 mg/kg bw per day). The NOAEL for reproductive toxicity was 5000 ppm (equal to 278 mg/kg bw per day), the highest dose tested.

In a developmental toxicity study in rats, increased early resorptions and post-implantation loss and decreased live young per litter and litter weight were observed when pregnant rats were dosed at 1000 mg/kg bw per day. Reductions in body weight and feed consumption were observed in maternal animals at this dose. The NOAEL for maternal and developmental toxicity in rats was 250 mg/kg bw per day. In rabbits, there was one abortion at the high dose (225 mg/kg bw per day), which occurred in the presence of a marked reduction in feed consumption and body weight in that dam. Litter and fetal weights were also reduced at the high dose, resulting in decreased gravid uterine weight. The NOAEL for maternal and developmental toxicity in rabbits was 75 mg/kg bw per day.

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

In an acute neurotoxicity study, the NOAEL was 125 mg/kg bw based on clinical signs of neurotoxicity at doses of 500 mg/kg bw (decreased motor activity and body temperature, unsteady gait, hunched posture); however, there was no histological evidence of damage to the central or peripheral nervous system. There was no evidence of neurotoxicity in the 90-day neurotoxicity study. A developmental neurotoxicity study revealed no maternal effects at 500 mg/kg bw per day, the highest dose tested. In contrast, the NOAEL for offspring toxicity was 100 mg/kg bw per day, based on decreased body weight at doses of 250 mg/kg bw per day and higher.

In a 4-week immunotoxicity study in mice, the NOAEL for immunotoxicity was 250 mg/kg bw per day, based on decreased plaque-forming cells in the spleen at 1000 mg/kg bw per day. In a 4-week immunotoxicity study in rats, no adverse effects were observed at any dose up to 700 mg/kg bw per day, the highest dose tested.

Toxicological data on metabolites

A variety of metabolites were also assessed for toxicity. These are minor metabolites in rats that are also found in livestock, plants and soil. The oral LD_{50} in rats for the metabolite DM-PCA was greater than 2000 mg/kg bw. In a 90-day feeding study in rats, the NOAEL for DM-PCA was 4000 ppm (equal to 258 mg/kg bw per day), based on reduced body weight gain and feed consumption at 16 000 ppm (equal to 1038 mg/kg bw per day). DM-PCA was not genotoxic in any of an adequate range of in vitro genotoxicity assays.

The oral LD_{50} in rats for the metabolite PCA was greater than 2000 mg/kg bw. In a 28-day oral gavage study of PCA in rats, the NOAEL was 1000 mg/kg bw per day, the highest dose tested. PCA was not genotoxic in any of an adequate range of in vitro and in vivo genotoxicity assays. The metabolite PAM was more acutely toxic than the parent and PCA, with an LD_{50} estimated between 300 and 2000 mg/kg bw by the oral route in rats. PAM was negative in the Ames assay but was positive without activation in a mouse lymphoma assay, in which small colony mutant frequencies were

increased. It induced chromosomal aberrations in mammalian cells in the absence of activation in vitro, but this clastogenicity was not confirmed in an in vivo micronucleus assay. Overall, the weight of evidence suggests that PAM has low potential for genotoxicity in vivo.

The oral LD_{50} in rats for the metabolite 753-A-OH was greater than 2000 mg/kg bw. The acute oral toxicity of the metabolite 753-T-DO has not been assessed. Neither 753-T-DO nor 753-A-OH was genotoxic in three in vitro assays to assess gene mutations and chromosomal aberrations.

The metabolites were not considered to be more toxic than penthiopyrad, with the exception of PAM, which was more acutely toxic than penthiopyrad and was genotoxic in vitro, but not in vivo.

There were no reports of adverse health effects in manufacturing plant personnel or in operators and workers exposed to penthiopyrad formulations during their use. Also, there was no evidence to support any findings in relation to poisoning with penthiopyrad.

The Meeting concluded that the existing database on penthiopyrad 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 11 mg/kg bw per day in the multigeneration reproduction study in rats for decreased body weight gain in F_1 males and adrenal effects in F_1 females (increased weight and cortical hypertrophy). A safety factor of 100 was applied.

The Meeting established an acute reference dose (ARfD) of 1 mg/kg bw on the basis of a NOAEL of 125 mg/kg bw in the acute neurotoxicity study in rats for clinical signs of neurotoxicity (e.g. decreased motor activity and body temperature, hunched posture, unsteady gait). 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	60 mg/kg bw per day	200 mg/kg bw per day
		Carcinogenicity	600 mg/kg bw per day[b]	—
Rat	Two-year study of toxicity and carcinogenicity[a]	Toxicity	27 mg/kg bw per day	83 mg/kg bw per day
		Carcinogenicity	83 mg/kg bw per day	250 mg/kg bw per day
	Two-generation study of reproductive toxicity[a]	Parental toxicity	11 mg/kg bw per day	54 mg/kg bw per day
		Offspring toxicity	54 mg/kg bw per day	278 mg/kg bw per day
		Reproductive toxicity	278 mg/kg bw per day[b]	—
	Developmental toxicity study[c]	Maternal toxicity	250 mg/kg bw per day	1000 mg/kg bw per day
		Embryo and fetal toxicity	250 mg/kg bw per day	1000 mg/kg bw per day
	Acute neurotoxicity study[c]	Neurotoxicity	125 mg/kg bw	500 mg/kg bw
Rabbit	Developmental toxicity study[c]	Maternal toxicity	75 mg/kg bw per day	225 mg/kg bw per day
		Embryo and fetal toxicity	75 mg/kg bw per day	225 mg/kg bw per day
Dog	Thirteen-week and 1-year studies of toxicity[a,d]	Toxicity	3000 ppm, equal to 76.7 mg/kg bw per day	15 000 ppm, equal to 445 mg/kg bw per day

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

Estimate of acceptable daily intake for humans

0–0.1 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 penthiopyrad

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid; ~90%
Distribution	Widely distributed; highest concentrations in liver
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%)
Potential for accumulation	No evidence of accumulation
Metabolism in mammals	Extensive
Toxicologically significant compounds (animals, plants and the environment)	Parent compound, PAM
Acute toxicity	
Rat LD_{50}, oral	> 2000 mg/kg bw
Rat, LD_{50}, dermal	> 2000 mg/kg bw
Rat, LC_{50}, inhalation (whole-body exposure)	> 5.7 mg/l
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Minimally irritating
Guinea-pig, dermal sensitization (Magnusson & Kligman)	Not sensitizing
Short-term studies of toxicity	
Target/critical effect	Liver (clinical chemistry changes), thyroid (increased weights, hypertrophy), adrenal (increased weights, hypertrophy)
Lowest relevant oral NOAEL	40 mg/kg bw per day (90-day study in rats)
Lowest relevant dermal NOAEL	1000 mg/kg bw per day (28-day study in rats)
Lowest relevant inhalation NOAEC	No data
Genotoxicity	
	No evidence for genotoxic potential
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Body weight, liver (periportal fatty degeneration)
Lowest relevant oral NOAEL	27 mg/kg bw per day (2-year study in rats)
Carcinogenicity	Unlikely to pose a carcinogenic risk to humans at anticipated dietary exposure levels

Reproductive toxicity	
Reproduction target/critical effect	No effect on fertility at highest dose tested; decrease in body weight in pups and slight delay in sexual maturation at parentally toxic dose
Lowest relevant reproductive NOAEL	278 mg/kg bw per day (highest dose tested) for reproductive effects (rats)
	11 mg/kg bw per day for systemic toxicity in parent (rats)
	54 mg/kg bw per day for offspring toxicity (decreased body weight) (rats)
Developmental target/critical effect	Decreased fetal weight at maternally toxic dose
Lowest relevant developmental NOAEL	75 mg/kg bw per day (rabbits)
Neurotoxicity/delayed neurotoxicity	
Neurotoxicity target/critical effect	Decreased motor activity, hunched posture, unsteady gait
Lowest relevant neurotoxicity NOAEL	125 mg/kg bw (acute neurotoxicity study, rats)
Immunotoxicity	
	Not immunotoxic (mice and rats)
Medical data	
	No data

Summary

	Value	Study	Safety factor
ADI	0–0.1 mg/kg bw	Rat, two-generation reproduction study	100
ARfD	1 mg/kg bw	Rat, acute neurotoxicity study	100

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PROPYLENE OXIDE

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Explanation

Propylene oxide is the International Organization for Standardization (ISO)–approved name for methyloxirane (International Union of Pure and Applied Chemistry) (Chemical Abstracts Service No. 75-56-9). Propylene oxide is a highly reactive, volatile compound (boiling point 34 ºC) that is used, as a gas or pressurized liquid, for fumigation and sterilization to control insect infestations and microbial spoilage in a range of food commodities (e.g. herbs, spices and nuts). The primary residues detected after propylene oxide use are propylene oxide, propylene chlorohydrin (chloropropanol), propylene bromohydrin (bromopropanol) and propylene glycol.

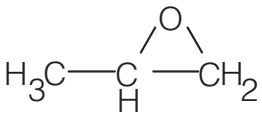
Propylene oxide was reviewed for the first time by the Joint FAO/WHO Meeting on Pesticide Residues at the request of the Codex Committee on Pesticide Residues.

The database for propylene oxide and propylene chlorohydrin consists mainly of published papers, often with limited levels of detail and no statements of compliance with good laboratory practice.

The conversion factor for propylene oxide in air is: 1 part per million (ppm) of propylene oxide is equal to 2.4 mg/m^3 at 25 °C (ECB, 2002).

The structure of propylene oxide is shown in Figure 1.

Figure 1. Structure of propylene oxide



Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

(a) Oral route

No data were submitted.

(b) Dermal route

No data were submitted.

(c) Inhalation route

A review by the USEPA (2006) stated that, like ethylene oxide, propylene oxide will probably be completely absorbed, distributed throughout the body and rapidly metabolized following inhalation.

A review by ECB (2002) described a study (Nolan et al., 1980) demonstrating that male rats exposed to propylene oxide by inhalation (6 hours; 80–904 ppm) had low blood concentrations of propylene oxide (levels not stated), but there was a disproportionate increase in blood levels above 143 ppm, indicating a saturation of metabolism and/or elimination. This is contrary to the findings of another study (Golka et al., 1989), also reviewed in ECB (2002), which is cited as reporting no saturation at up to 3000 ppm.

Male F344 rats (five per group) were exposed to propylene oxide (99.99% pure; Lyondell Chemicals) by inhalation, 6 hours/day, for 1 day (10–750 ppm), 1–5 days (50 or 100 ppm) or 3–28 days (5 days/week; 5–500 ppm). At sacrifice, blood samples were taken within 2 minutes, and nasal tissues, liver and major organs were removed. Analyses were performed for propylene oxide levels in blood and for non-protein sulfhydryls in liver, blood, lung and nasal tissues. Single exposures gave a relationship between propylene oxide concentrations in blood and air, with an air concentration of 300 ppm propylene oxide equating to a concentration of approximately 12.5 µmol/l in blood. Repeated exposures indicated a non-linear increase in propylene oxide concentrations in blood at air concentrations above 300 ppm. Non-protein sulfhydryl groups were reduced by more than 50% in nasal mucosa, lung and liver after single exposures to propylene oxide at concentrations of 300 ppm and above. After repeated exposures, the reductions in non-protein sulfhydryls were less marked in liver and lung, but still marked in nasal mucosa (Lee et al., 2005).

A physiologically based pharmacokinetic model was developed based on the results of Lee et al. (2005). The primary focus was to provide a comparison of human and rat exposures to propylene oxide following inhalation. The model indicated that systemic exposures and local concentrations in the nasal mucosa would be similar in rats and humans following inhalation exposure (Csanady & Filser, 2007).

There are no reliable data that would permit a reliable conversion of an inhalation exposure level to a systemic dose. For the purposes of this assessment, a simplistic conversion between inhalation exposures and oral dosing has been performed. For rats, this conversion assumed standard breathing rates and volumes, a body weight of 250 g and 100% absorption via each exposure route. The conversion resulted in an atmospheric concentration of 100 ppm (240 mg/m^3) inhaled for 6 hours/day, 5 days/week, being approximately equivalent to an oral dose of 40 mg/kg body weight (bw) per day in rats. The corresponding value for mice is considered to be approximately double that for rats, giving 100 ppm equivalent to 80 mg/kg bw per day. These are likely to be conservative estimates for systemic propylene oxide exposures via the oral route.

1.2 Biotransformation

Based on in vitro hydrolysis data, propylene oxide is predicted to hydrolyse very rapidly, with a half-life for hydrolysis of approximately 1 minute under the conditions found in the mammalian stomach (pH 1, 37 ºC) (Meylan et al., 1986). This rapid hydrolysis was confirmed for human synthetic gastric juice (pH 1.46), with a half-life of approximately 2 minutes, but not for rat synthetic gastric juice (pH 4.8), in which the half-life was estimated to be greater than 2 hours (Todhunter, 2000). With human blood samples in vitro, the half-life was cited as being 13.6 hours (SCOEL, 2009).

A review by ECB (2002) described a study indicating that male rats exposed to propylene oxide by inhalation (6 hours; > 217 ppm) had reduced levels of hepatic non-protein sulfhydryl groups. Depression of lung and kidney sulfhydryl groups was observed at 625 ppm. This indicates a possible role for glutathione conjugation in the metabolism of propylene oxide. This review also cited the authors of a 1989 paper (Golka et al., 1989) who deduced that 96% of inhaled propylene oxide was metabolized, although the underlying reasoning is unknown.

An in vitro study with Sprague-Dawley rat liver microsomes showed that propylene oxide was a substrate for epoxide hydrolase, being hydrolysed to 1,2-propylene glycol. Of all the epoxides tested, the rate of hydrolysis was slowest for propylene oxide (Guengerich & Mason, 1980).

The limited data available indicate that the majority of propylene oxide ingested is unlikely to be systemically available. Ingested propylene oxide is likely to be hydrolysed to propylene chlorohydrin or propylene glycol in the acidic conditions of the stomach. Any absorbed propylene oxide will be extensively metabolized to propylene glycol in the liver or bound to sulfhydryl groups.

Table 1. Acute toxicity studies with propylene oxide

Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	LC_{50} (mg/l)	Purity (%); batch	Vehicle	Reference
Mouse	—	—	Oral	630	—	—	Not stated	Antonova et al. (1981)
Rat	—	—	Oral	300–1000	—	—	Not stated	Rowe et al. (1956)
Rat	—	—	Oral	950	—	—	Not stated	Smyth et al. (1969)
Rat	—	—	Oral	520	—	—	Not stated	Antonova et al. (1981)
Guinea-pig	—	—	Oral	660	—	—	Not stated	Antonova et al. (1981)
Guinea-pig	—	—	Oral	690	—	—	Not stated	Smyth, Seaton & Fischer (1941)
Rabbit	—	—	Dermal	1250	—	—	Not stated	Weil et al. (1963)
Rabbit	—	—	Dermal	950	—	—	Not stated	Smyth et al. (1969)
Mouse	—	—	Inhalation 4 h	—	4.1	—	None	Jacobson, Hackley & Feinsilver (1956)
Mouse	—	—	Inhalation 4 h	—	1.0–1.5[a]	—	None	NTP (1985); USEPA (2006)
Rat	—	—	Inhalation 4 h	—	9.5	—	None	Weil et al. (1963)
Rat	—	—	Inhalation 0.5 h	—	17	—	None	Jacobson, Hackley & Feinsilver (1956)
Rat	—	—	Inhalation 4 h	—	3.2–3.4[a]	—	None	NTP (1985); USEPA (2006)

LC_{50}, median lethal concentration; LD_{50}, median lethal dose
[a] Values calculated by USEPA (2006) based on data in NTP (1985).

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

Propylene oxide was of moderate acute toxicity via the oral and dermal routes and of moderate acute toxicity via the inhalation route (Table 1).

(b) Dermal and ocular irritation and dermal sensitization

A review by ECB (2002) presented conflicting results for the skin irritating properties of propylene oxide. In one study in rabbits, propylene oxide was considered to be non-irritating following a 4-hour exposure, but in another study, severe erythema and oedema progressing to scar tissue were reported in rabbits exposed for longer than 6 minutes. Propylene oxide vapour is reported to be irritating to the eye and respiratory tract (ECB, 2002). A review by USEPA (2006) categorized propylene oxide as a severe irritant to both skin and eyes.

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

No data were available.

Rats

In a 1956 study cited by ECB (2002), a no-observed-adverse-effect level (NOAEL) of 200 mg/kg bw per day was reported for rats (five of each sex per group) administered propylene oxide by

gavage 18 times over 24 days. Reduced body weight gain, increased gastric irritation and unspecified hepatotoxicity were seen at 300 mg/kg bw per day (Rowe et al., 1956).

In a 1981 study, a NOAEL of 0.0052 mg/kg bw per day was reported for rats administered propylene oxide in drinking-water for 26 weeks. At 0.052 mg/kg bw per day, mild haematological effects were reported. At the highest dose of 0.52 mg/kg bw per day, polyuria, haematological changes, altered serum protein levels and increased activity of intestinal mucosa enzymes were reported (Antonova et al., 1981).

The reason for the apparent difference in NOAELs in these studies (4 orders of magnitude) is unknown. The results in the Antonova et al. (1981) paper were inconsistent with other data on propylene oxide, and the methodology was poorly described. The Meeting decided not to use the findings in the risk assessment for propylene oxide.

Dogs

No data were available.

(b) Dermal application

No data were available.

(c) Exposure by inhalation

A number of published studies have been summarized in reviews by IPCS (1985), ECB (2002) and USEPA (2006). The most appropriate reports are considered to be those from the United States National Toxicology Program (NTP, 1985), which are summarized below.

Mice

Groups of B6C3F1 mice (five of each sex per concentration) were exposed to propylene oxide (99.9% pure; lot No. UC 5/10/76) in an inhalation chamber for 6 hours/day, 10 times over 12 days. Concentrations in the chamber were 0, 20, 47, 99, 196 and 487 ppm. Animals were observed daily, weighed on days 0, 4 and 8 and at termination and subjected to a terminal gross necropsy. No histopathological examination was performed. The only finding reported was dyspnoea at 196 and 487 ppm.

Within the limitations of the study protocol, the no-observed-adverse-effect concentration (NOAEC) was 99 ppm (~240 mg/m^3), based on dyspnoea at 196 ppm (NTP, 1985).

Groups of B6C3F1 mice (10 of each sex per concentration) were exposed to propylene oxide (99.9% pure; lot No. UC 5/10/76) in an inhalation chamber for 6 hours/day, 5 days/week, for 13 weeks (63 exposures). Mean time-weighted concentrations in the chamber were 0, 31, 63, 125, 250 and 500 ppm. Animals were observed daily, weighed weekly and subjected to a terminal gross necropsy. Histopathological examination was performed on a wide range of tissues from control and 500 ppm animals.

One male from the 125 ppm group died. Body weight gains were depressed approximately 40–55% at 500 ppm. No adverse effects were reported at either gross or microscopic examination.

A NOAEC of 250 ppm (600 mg/m^3) can be determined, based on the body weight effects at 500 ppm (NTP, 1985).

Rats

Groups of F344/N rats (five of each sex per concentration) were exposed to propylene oxide (99.9% pure; lot No. UC 5/10/76) in an inhalation chamber for 6 hours/day, 10 times over 12 days. Concentrations in the chamber were 0, 47, 99, 196, 487 and 1433 ppm. Animals were observed daily, weighed on days 0, 4 and 8 and at termination and subjected to a terminal gross necropsy. No histopathological examination was performed. One male exposed at 1433 ppm died. Rats exposed at

1433 ppm lost weight (males) or failed to gain weight (females) and exhibited dyspnoea, hyperactivity, gasping ataxia and diarrhoea.

Within the limitations of the study protocol, the NOAEC was 487 ppm (~1200 mg/m^3), based on body weight loss and clinical signs at 1433 ppm (NTP, 1985).

Groups of F344/N rats (10 of each sex per concentration) were exposed to propylene oxide (99.9% pure; lot No. UC 5/10/76) in an inhalation chamber for 6 hours/day, 5 days/week, for 13 weeks (62 exposures). Mean time-weighted concentrations in the chamber were 0, 31, 63, 125, 250 and 500 ppm. Animals were observed daily, weighed weekly and subjected to a terminal gross necropsy. Histopathological examination was performed on a wide range of tissues from control and 500 ppm animals.

There were no deaths. Body weight gains were depressed approximately 15% at 500 ppm. No adverse effects were reported at either gross or microscopic examination.

A NOAEC of 250 ppm (600 mg/m^3) can be determined, based on the body weight effects at 500 ppm (NTP, 1985).

2.3 Long-term studies of toxicity and carcinogenicity

(a) Oral administration

Mice

No data were available.

Rats

Female SD rats (50 per group) received propylene oxide (99% pure; Merck-Schuschardt) by gavage at a dose level of 15 or 60 mg/kg bw twice per week for up to 150 weeks (equal to 4.3 and 17 mg/kg bw per day). Both untreated, vehicle (salad oil) and β-propiolactone control groups were included. At necropsy, gross examinations were performed, and "pathologically noteworthy" organs were fixed, stained and examined histopathologically.

Survival was similar in untreated, vehicle and propylene oxide groups, with greater than 50% survival until approximately week 105. Within the limitations of the investigative procedure, the only organ with an increased incidence of non-neoplastic lesions and tumours was the stomach/forestomach (data not presented separately). The tumour incidence in the stomach/forestomach showed a clear dose–response relationship and is shown in Table 2. Only the most severe finding is presented in the results in the paper; that is, an animal with hyperkeratosis and carcinoma would be recorded only under the carcinoma heading. This makes it impossible to determine any association between hyperkeratosis and tumours or the absolute incidence of hyperkeratosis.

With the limited level of tissue investigation and data presentation, this study is unable to demonstrate a NOAEL for chronic toxicity because of the presence of hyperkeratosis at 4.3 mg/kg bw per day, the lowest dose tested. The lowest dose level had a slight, but not statistically significant, increase in squamous cell carcinoma of the stomach, compared with 0 in 100 control animals. The NOAEL for carcinogenicity could not be determined and would be less than 4.3 mg/kg bw per day (Dunkelberg, 1982).

(b) Exposure by inhalation

Mice

Groups of B6C3F1 mice (50 of each sex per concentration) were exposed to propylene oxide (99.9% pure; lot No. 64772) in an inhalation chamber for 6 hours/day, 5 days/week, for 2 years. Mean

Table 2. Stomach tumour and lesion incidences in female rats exposed to propylene oxide

Tumour type	Incidence of finding		
	Controls (untreated and vehicle; n = 100)	Propylene oxide (15 mg/kg bw; n = 50)	Propylene oxide (60 mg/kg bw; n = 50)
Squamous cell carcinoma	0	2	19
Adenocarcinoma	0	0	1
Carcinoma in situ	0	0	1
Hyperkeratosis/hyperplasia/papilloma	—	7	17
Animals with stomach tumours	0	2	20

From Dunkelberg (1982)
— incidence not specified

time-weighted concentrations in the chamber were 0, 200 (495 exposures) and 400 ppm (491 exposures). Animals were observed daily, weighed weekly and subjected to a terminal gross necropsy. Histopathological examination was performed on a wide range of tissues from all animals.

Survival was reduced in both treated groups during the second half of the study (Table 3). The survival in the 400 ppm females became less than 50% at week 88 and might have compromised the ability to investigate carcinogenic potential. Body weights were significantly lower in the 400 ppm groups during the second half of the study. No compound-related clinical signs were reported. Inflammation of the nasal epithelia was seen in all propylene oxide–treated groups (Table 3). Low incidences of squamous cell carcinoma and adenocarcinoma were present in high-dose animals; both tumours were described as arising from the submucosal glands and spread along the nerve sheaths. There was also an increase in haemangiosarcoma and haemangioma of the vascular plexus below the nasal epithelium (Table 3). These nasal tumours had not been reported in previous studies, but the degree of examination, involving three levels of the nasal turbinates, is much greater than in many other NTP mouse studies in which the turbinates were not always examined or were examined only at one level. An increase in mammary gland adenocarcinoma was seen in females, which was statistically significant in the high-dose group when corrected for survival; the incidences are within the historical control range and considered to be not clearly treatment related.

A NOAEC for site of contact toxicity cannot be derived for this study due to the inflammation of the nasal epithelia seen at both concentrations. The NOAEC for carcinogenicity was 200 ppm (480 mg/m^3), based on the nasal tumours seen at 400 ppm. The NOAEC for systemic toxicity was 200 ppm, based on reduced body weight gain at 400 ppm (NTP, 1985).

Rats

Wistar rats (70 of each sex per group) were exposed to propylene oxide (> 99.9% pure, Dow Chemicals) at 0, 30, 100 or 300 ppm in the air for 6 hours/day, 5 days/week, for up to 28 months. Additional satellite groups of 10 rats of each sex were sacrificed after 12, 18 or 24 months. Animals were housed in whole-body inhalation chambers. Routine observations of morbidity, mortality and body weight were performed. Samples for haematological, clinical chemistry and urine analyses were taken at sacrifice of the satellite group animals. A gross pathological examination was performed on all animals. Major organs from all satellite animals and 20 of each sex per group at terminal sacrifice were weighed. Microscopic histopathological examinations were performed on a wide range of tissues from all control and 300 ppm animals from the 12- and 24-month satellite groups and animals killed at study termination. A limited histopathological examination, concentrating on the respiratory tract and tumours, was performed on the 18-month satellite groups and intermediate-exposure groups at the end of the study. Lungs were examined in control and 300 ppm groups only at termination.

Table 3. Findings in mice exposed to propylene oxide by inhalation for 2 years

	Incidence of finding (n = 50)					
	0 ppm		200 ppm		400 ppm	
	Males	Females	Males	Females	Males	Females
Survival to termination (%)	*84*	*74*	*68*	*58*	*54*	*10*
Body weight (week 90) (g)	*38*	*32*	*37*	*30*	*30**	*27**
Nasal epithelia suppurative inflammation	0	0	8*	16*	4	23*
Nasal epithelia acute/chronic inflammation	0	0	14*	14*	38*	18*
Nasal epithelia squamous metaplasia	0	0	1	0	0	2
Nasal epithelia squamous cell carcinoma (HC)	0 (0)	0 (0)	0	0	1	0
Nasal epithelia adenocarcinoma (HC)	0 (0)	0 (0)	0	0	0	2
Nasal cavity haemangiosarcoma (HC)	0 (0)	0 (0)	0	0	5*	2
Nasal cavity haemangioma (HC)	0 (0)	0 (0)	0	0	5*	3
Mammary gland adenocarcinoma (HC)	—	0 (0–12%; 2.4%)	—	3	—	3

From NTP (1985)
HC, historical control range and mean; * $P < 0.05$

Mean measured air concentrations were within 3% of nominal. Survival was greater than 50% in all groups at week 105, but subsequently there was a statistically significant ($P < 0.05$) increase in deaths in the 300 ppm groups from week 115 and in 100 ppm females at week 119. Body weights were reduced in the 300 ppm groups. There were no consistent effects on clinical chemistry, urinalysis or organ weights that were related to propylene oxide exposure. Increases in relative liver weights (10–15%) were statistically significant at 300 ppm in males sacrificed at 24 and 28 months and in females sacrificed at 24 months. Body weights were reduced slightly (< 5%) in treated groups during the initial stages of the study, but not at termination; this is not considered to be biologically relevant. There were indications of increased leukocyte counts in samples taken after 100 weeks from animals exposed at 300 ppm. Local effects on the basal mucosa, nasal turbinates and olfactory epithelium were seen at 300 ppm and occasionally at 100 ppm from 12 months onwards (Table 4). Non-neoplastic findings were seen in the heart, liver, lung and kidneys at 300 ppm; the effects at 100 and 30 ppm are unclear due to the limited number of tissues examined (Table 4). There were no increases in tumour incidence in the nose or respiratory tract. Increased incidences of a number of tumours of the mammary glands and thyroid were recorded in the main groups of animals (Table 4). The incidences of multiple mammary gland tumours were increased in all treated female groups, but were reported to be within historical control ranges.

The NOAEC for systemic toxicity was 100 ppm, based on body weight gain reductions at 300 ppm. The increased mortality at 100 ppm after week 115 is not considered relevant, as this is beyond the typical lifespan of laboratory rats. The NOAEC for tumours was 300 ppm, the highest dose tested (Reuzel & Kuper, 1983).

Groups of F344/N rats (50 of each sex per concentration) were exposed to propylene oxide (99.9% pure; lot No. 6477-22) in an inhalation chamber for 6 hours/day, 5 days/week, for 2 years (491 exposures). Mean time-weighted concentrations in the chamber were 0, 200 and 400 ppm. Animals were observed daily, weighed weekly and subjected to a terminal gross necropsy. Histopathological examination was performed on a wide range of tissues from all animals.

Survival was similar in all groups and greater than 50% at study termination. Body weights were slightly lower (< 10%) in the 400 ppm groups. Inflammation of the nasal cavity was increased

Table 4. Tumour and non-neoplastic lesion incidences in rats exposed to propylene oxide by inhalation

	Incidence of lesions (n = 70)						
	Males[a]			Females[b]			
	Control	100 ppm	300 ppm	Control	30 ppm	100 ppm	300 ppm
Deaths, week 109	*17*	*18*	*24*	*14*	—	*18*	*26**
No. surviving to scheduled sacrifice	*37*	*35*	*14**	*36*	—	*27*	*15**
Mammary gland mass / suspected tumour	7	3	3	33	—	46*	48*
Mammary gland (fibro)adenoma (HC 19–67%)	1	0	0	32	30	39	47*
Multiple fibroadenoma	0	0	0	2	8*	13*	22*
Mean fibroadenomas	0	0	0	1.3	2.1	2.2	2.4
Mammary gland tubulopapillary carcinoma	0	0	0	3	6	5	8*
Brain glial cell tumour	2	0	5	0	—	0	1
Thyroid follicular adenoma	1	0	5	1	—	0	7*
Thyroid parafollicular adenoma	3	1	6	6	—	0	10
Uterus endometrial polyp	—	—	—	9	—	14	18*
Rats with tumours	49	34	53	52	—	61	67
Rats with malignant tumours	19	22	34*	6	—	14	26*
Liver, hepatocellular necrosis	1	0	6*	0	—	1	4*
Myocardial degeneration	15	1	18	3	—	0	10*
Thrombi of the heart	2	4	9*	3	—	1	3
Dilated renal tubules	9	0	11	0	—	3	7*
Olfactory epithelia basal cell hyperplasia (12 months, n = 10)	0	2	5*	0	—	0	7*
Olfactory epithelia basal cell hyperplasia (termination, n = 70)	4	10	24*	0	—	9*	33*
Alveolar bronchiolization	5	2	10	3	—	0	7

From Reuzel & Kuper (1983)

* $P < 0.05$

[a] No data for 30 ppm are presented, as there were no notable findings.

[b] Most data for 30 ppm are not presented, as there were few notable findings.

at 400 ppm and in males at 200 ppm (Table 5). Tumours of the nasal cavity (papillary adenoma) were increased in both sexes at 400 ppm, outside the historical control range. Other tumours showing increased incidences were mammary gland, uterus and thyroid tumours in females (Table 5). The uterine stromal sarcoma incidences did not exhibit a dose–response relationship, but were above the historical control range at both concentrations of propylene oxide. The thyroid gland C-cell tumours were at the upper end of the historical control range, and as there was no related increase in hyperplasia, the relationship to propylene oxide is considered equivocal. The mammary gland tumours are consistent with results in other studies but are not statistically significant and are within the historical control range; their relationship to propylene oxide is equivocal.

The NOAEC for tumours was 200 ppm, based on the increase in papillary adenomas of the nasal cavity at 400 ppm. The NOAEC for systemic toxicity was 200 ppm, based on reduced body weight gain at 400 ppm. A NOAEC for chronic site of contact toxicity could not be determined and would be less than 200 ppm, based on nasal cavity inflammation (NTP, 1985).

Table 5. Findings in rats exposed to propylene oxide by inhalation for 2 years

	Incidence of finding (n = 50)					
	0 ppm		200 ppm		400 ppm	
	Males	Females	Males	Females	Males	Females
Survival to termination (%)	*60*	*70*	*66*	*64*	*62*	*64*
Body weight (week 90) (g)	*442*	*309*	*435*	*291*	*409*	*284*
Nasal cavity suppurative inflammation	7	3	19*	5	33*	20*
Nasal epithelia hyperplasia	0	1	1	0	11*	5
Nasal epithelia squamous metaplasia	0	1	3	2	21*	11*
Nasal cavity papillary adenoma (HC)	0 (1/1477)	0 (3/1523)	0	0	2	3
Mammary gland fibroadenoma	0	7	2	13	2	13
Mammary gland adenocarcinoma	0	1	0	1	0	1
Thyroid C-cell adenoma/ carcinoma (HC)	1	2 (mean 8%; range 2–18%)	2	2	4	7*
Uterus endometrial stromal sarcoma (HC)	—	0 (0–2%)	—	4	—	2
Adrenal medulla focal hyperplasia	2	2	2	2	10*	3
Splenic fibrosis	4	0	8	8	5	6

From NTP (1985)
HC, historical control incidence; * $P < 0.05$

2.4 Genotoxicity

Testing of the genotoxicity of propylene oxide has been performed in a wide range of assays. The overall database is considered adequate to conclude that propylene oxide is an in vitro genotoxin and is a potential site of contact alkylating agent in vivo. The level of information in many of the studies was such that it was not possible to make an independent evaluation but necessary to rely on the conclusions of the authors.

Key data on the genotoxicity of propylene oxide are summarized in Table 6.

There are no in vivo genotoxicity data from tissues directly exposed to propylene oxide rather than its metabolites. Special investigative studies have reported that propylene oxide produces deoxyribonucleic acid (DNA) adducts (primarily N^7G, plus N^3A, N^3C and N^1A) in respiratory mucosa and liver of exposed rats (IARC, 2010; see also section 2.6).

2.5 Reproductive toxicity

(a) Multigeneration studies

No data are available via the oral route.

Mice

No data are available.

Table 6. Results of studies of genotoxicity with propylene oxide

Vehicle	End-point	Test object	Concentration	Purity (%); supplier	Result	Reference
In vitro						
Water, DMSO, 0.5% tragacanth	Reverse mutation	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538 *Escherichia coli* WP2*uvrA*, CM891, CM871	1.1–700 µg/plate, with and without S9 mix	99.5; BDH	Positive with TA100 and TA1535 (±S9); negative with other strains	Bootman, Lodge & Whaley (1979)
—	Reverse mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538, G46, C3076, D3052 *E. coli* WP2, WP2*uvrA*	2.5–20 µl/plate in volatile liquid apparatus; −S9	Not specified	Positive with TA100, TA1535 and *E. coli*	McMahon, Cline & Thompson (1979)
Water	Forward mutation	*Schizosaccharomyces pombe* P1	3, 10, 30 mmol/l (sealed tube); ±S9	Not specified; Merck	Positive (±S9)	Migliore, Rossi & Loprieno (1982)
Acetone	Reverse mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537	Up to 200 µmol/plate; −S9	99; Merck-Schuschardt	Positive with TA100 and TA1535	Pfeiffer & Dunkelberg (1980)
—	Reverse mutation	Chinese hamster ovary (CHO-K_1-BH_4) cells	8–40 µg/cm^3 (protocol optimized for volatile compounds) (1 h)	Not specified; Kodak Chemical Co.	Positive at ≥ 8 µg/cm^3	Zamora et al. (1983)
—	Sister chromatid exchange	Human lymphocytes (two non-smoking donors)	2.5% in air (30–300 s)	Not specified; Matheson Gas	Positive at 100 and 300 s; negative at 30 s	Tucker et al. (1986)
Water	Chromosomal aberration	Human lymphocytes (two male donors)	1.85, 9.25 µg/ml; −S9 (24 h)	99.5; BDH	Positive	Bootman, Lodge & Whaley (1979)
Water or DMSO	Chromosomal aberration	Rat liver epithelial cells	25, 50, 75, 100 µg/ml; ±S9 (24 h)	Not specified; Shell Chemicals, United Kingdom	Positive (±S9)	Dean & Hodson-Walker (1979)
Methanol	DNA base reactivity	Pure chemicals from calf thymus	0.5 nmol/mg (deoxyadenosine; 68 h) 0.5 nmol/mg (deoxyguanosine; 24 h) 0.2 nmol/mg (DNA)	Not specified; Aldrich Chemical Co.	Positive	Djuric et al. (1986)

Table 6 (continued)

Vehicle	End-point	Test object	Concentration	Purity (%); supplier	Result	Reference
In vivo						
0.5% gum tragacanth	Micronucleus assay	CD-1 mouse bone marrow (gavage)	0, 100, 250, 500 mg/kg bw, twice (30 h)	99.5; BDH	Negative	Bootman, Lodge & Whaley (1979)
0.5% gum tragacanth	Micronucleus assay	CD-1 mouse bone marrow (intraperitoneal)	0, 75, 150, 300 mg/kg bw, twice (30 h)	99.5; BDH	Positive at 300 mg/kg bw	Bootman, Lodge & Whaley (1979)
0.5% gum tragacanth	Dominant lethal	Male CD-1 mouse (gavage)	0, 50, 250 mg/kg bw per day for 14 days	99.5; BDH	Negative	Bootman, Lodge & Whaley (1979)
—	Dominant lethal	Male rats	300 ppm in air; 7 h/day for 5 days prior to mating	98; Eastman Kodak	Negative	Hardin et al. (1983a)
—	Sperm head morphology	Male mice	300 ppm in air (720 mg/m^3); 7 h/day for 5 days; investigations on days 1, 3, 5, 7 and 9 post-exposure	98; Eastman Kodak	Negative	Hardin et al. (1983a)
—	Sex-linked recessive lethal	*Drosophila melanogaster*	645 ppm in air (1550 mg/m^3); 24 h	98; Eastman Kodak	Positive	Hardin et al. (1983a)

DMSO, dimethyl sulfoxide; DNA, deoxyribonucleic acid; S9, 9000 × *g* supernatant from livers of rats

Rats

Groups of F344 rats (30 of each sex per group) were exposed to propylene oxide (> 99.7% pure) in whole-body inhalation chambers in a two-generation reproduction study. Exposures were at 0, 30, 100 or 300 ppm, 6 hours/day, 5 days/week, during pre-mating and 7 days/week from mating to weaning. Exposures started 14 weeks prior to first mating. Offspring of the first mating were selected and exposed in a similar manner to give the F_2 generation.

There were no effects reported on mating performance, fertility, litter size, pup survival or development. Reduced body weight gain was seen in parental animals and pups at 300 ppm. There were no adverse effects reported on gross or histopathological examination of pups from control or 300 ppm groups.

The NOAEC for reproductive toxicity was 300 ppm (720 mg/m^3), the highest dose tested. The NOAEC for parental and pup toxicity was 100 ppm (240 mg/m^3), based on reduced body weight gain at 300 ppm (Hayes et al., 1988).

(b) Developmental toxicity

No data are available via the oral route.

Mice

No data are available.

Rats

Groups of 25 mated Fischer 344 rats were exposed to propylene oxide at 0, 100, 300 or 500 ppm for 6 hours/day on days 6–15 of gestation. Dams were killed and caesarean sections performed on day 20 of gestation. Fetuses were removed and examined for external, skeletal and visceral abnormalities.

There were no adverse effects on survival, appearance or behaviour. Maternal body weight gain was reduced at 500 ppm. There was no increase in malformations, and the NOAEC for teratogenicity was 500 ppm. There were no effects on litter size, post-implantation losses, fetal viability or litter size. The only significant developmental finding was an increase in accessory cervical ribs at 500 ppm.

The NOAEC for developmental effects was 500 ppm (1200 mg/m^3), and the NOAEC for maternal effects was 300 ppm (720 mg/m^3) (Harris et al., 1989).

Mated female SD rats (32–45 per group) were exposed to propylene oxide (> 99% pure) via inhalation at 500 ppm for 7 hours/day. Groups of animals were exposed on days 7–16 of gestation, on days 1–16 of gestation or from 3 weeks pre-mating until day 16 of gestation, although it is not clear which dosing regimen applied to which results. On day 21 of gestation, dams were sacrificed, uterine contents removed and fetuses examined for external, visceral and skeletal abnormalities.

There were no deaths. Body weight gain was reduced in treated animals, whereas kidney, liver, lung and spleen weights were increased. There were decreases reported in numbers of corpora lutea and implantation sites and live fetus weights, length and numbers in the group exposed pre-mating and during gestation. Increased incidences of wavy ribs and reduced ossification of the ribs and vertebrae were reported for the exposed groups, possibly secondary to maternal toxicity. There were no increases in malformations.

The single air concentration tested (500 ppm; 1200 mg/m^3) was the NOAEC for teratogenicity, but a lowest-observed-adverse-effect concentration (LOAEC) for fetotoxicity and maternal toxicity (Hardin et al., 1983b).

Rabbits

Mated female New Zealand White rabbits (23–30 per group) were exposed to propylene oxide (> 99% pure) via inhalation at 500 ppm for 7 hours/day. Groups of animals were exposed on days 7–19 of gestation or days 1–19 of gestation. On day 30 of gestation, dams were sacrificed, uterine contents removed and fetuses examined for size, weight, viability and external, visceral and skeletal abnormalities.

There were reductions reported in maternal body weight gain, histopathological changes to a number of organs and increases in resorptions and minor skeletal abnormalities. There were no reported increases in malformations.

The single air concentration tested (500 ppm; 1200 mg/m^3) was a NOAEC for teratogenicity, but a NOAEC could not be determined for maternal toxicity and fetotoxicity (Hardin et al., 1983b).

2.6 Special studies

(a) Adduct formation

A review by ECB (2002) cited a number of studies reporting that inhaled propylene oxide gives rise to DNA or haemoglobin adducts in a range of tissues and species, including humans. The predominant reactions are the hydroxypropylation of adenosine and guanosine bases and of histidine. In F344 rats exposed to propylene oxide (500 ppm, 6 hours/day, 5 days/week, for 4 weeks), increased levels of N^7-(2-hydroxypropyl) guanine were recorded (using gas chromatography–mass spectrometry) in respiratory mucosa (835 pmol/μg) and liver (35 pmol/μg) on the day of the last exposure; 3 days after the last exposure, levels were 593 and 23 pmol/g in respiratory mucosa and liver, respectively.

(b) Stability in gastric juice

The relative stability of propylene oxide in synthetic human and rat gastric juices was determined by Thilagar, Patil & Todhunter (2000). Propylene oxide (99% pure; batch No. I4621JS) and [^{14}C]-propylene oxide (batch No. 990119; final activity 185 kBq/ml) were incubated at 37 °C in rat or human gastric juices and analysed by high-performance liquid chromatography for the presence of propylene oxide or propylene glycol. Samples were taken at regular intervals between 10 seconds and 2 hours. The human gastric juice had a pH of 1.46, and the rat forestomach juice had a pH of 4.8. The results showed that in human gastric juice, approximately 15% of the propylene oxide had hydrolysed to propylene glycol in the first 10 seconds, and over 90% of the propylene oxide had hydrolysed within 1 hour. In contrast, in rat gastric juice, less than 5% of the propylene oxide had hydrolysed in the first minute, and less than 20% had hydrolysed in 90 minutes. These results indicate that exposures to propylene oxide, as opposed to its hydrolysis products, are likely to be significantly more prolonged in the rat stomach than in the human stomach.

(c) Studies on propylene chlorohydrin

A number of studies have been performed on the propylene oxide plant metabolite propylene chlorohydrin (chloropropanol), which are summarized in NTP (1998). The material tested in the NTP studies was batch No. B15, containing approximately 75% 1-chloro-2-propanol and 25% 2-chloro-1-propanol.

Absorption, distribution, metabolism and excretion

Following oral administration of 1-chloro-2-propanol (purity unknown) to rabbits at 140 mg/kg bw, 11% of the dose was excreted in urine as its glucuronide conjugate. In rats, 2-hydroxypropyl-mercapturic acid was identified as a urinary metabolite of orally administered 1-chloro-2-propanol.

Table 7. Acute toxicity studies with propylene chlorohydrin

Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	LC_{50} (mg/l)	Purity (%); batch	Vehicle	Reference
Rat	Carworth-Wistar	—	Oral	200–250	—	—	Not stated	Smyth, Seaton & Fischer (1941); Smyth et al. (1969)
Guinea-pig	—	—	Oral	720	—	—	Not stated	
Dog	—	—	Oral	~200	—	—	Not stated	FAO (1974)
Rabbit	New Zealand White	—	Dermal	500	—	—	Not stated	Smyth et al. (1969)
Rat	—	—	Inhalation 6 h	—	> 3.8 (1000 ppm)	—	None	Gage (1970)

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

Rats dosed orally for 4 days with 1-chloro-2-propanol (100 mg/kg bw per day) had *N*-acetyl-*S*-(2-hydroxypropyl)-cysteine and β-chlorolactate as major urinary metabolites. Cysteine conjugates were also identified after inhalation exposures (cited in NTP, 1998).

Lethal doses

Propylene chlorohydrin (chloropropanol) was of moderate acute toxicity via the oral and dermal routes and of moderate acute toxicity via the inhalation route (Table 7).

Dermal and ocular irritation and dermal sensitization

Propylene chlorohydrin is not irritating to the skin of rabbits (Smyth et al., 1969), but it is reported to be severely irritating to the eyes of rabbits (Carpenter & Smyth, 1946).

Short-term studies of toxicity

Groups of B6C3F1 mice (10 of each sex per dose) received 1-chloro-2-propanol (batch No. B15) in drinking-water at 0, 100, 330, 1000, 3300 or 10 000 mg/l for 14 days. All animals were necropsied and major organs weighed. An extensive range of tissues from control and top-dose animals and liver, pancreas, spleen and thymus from selected groups were examined histopathologically. Achieved doses were not reported.

One male from the 10 000 mg/l group died. Mice in the top-dose groups lost weight and had an approximately 70% reduction in water consumption. At 3300 mg/l, body weight gain was similar to that of controls, with water consumption reduced by 20–40%. Atrophy of the spleen was present in top-dose groups. Pancreatic acinar cell alterations together with pancreatic degeneration and bone marrow atrophy were present in both sexes at and above 3300 mg/l. Hepatocyte vacuolation (mainly periportal) incidence was increased significantly at 1000 mg/l and above, with non-significant increases at 330 mg/l and in males at 100 mg/l.

A NOAEL of 330 mg/l (approximately 100 mg/kg bw per day, based on the values for the 14-week study) can be determined, based on the statistically significant increase in hepatocyte vacuolation in both sexes at 1000 mg/l (NTP, 1998).

Groups of B6C3F1 mice (10 of each sex per dose) received 1-chloro-2-propanol (batch No. B15) in drinking-water at 0, 33, 100, 330, 1000 or 3300 mg/l for 14 weeks. Achieved doses were reported to be 0, 5, 15, 50, 170 and 340 mg/kg bw per day in males and 0, 7, 20, 70, 260 and 340 mg/kg bw per day in females. All animals were necropsied and major organs weighed. A sample for haematology was taken at study termination. An extensive range of tissues from control and top-dose animals

Table 8. Findings in a 14-week mouse study with propylene chlorohydrin in drinking-water

	0 mg/l		33 mg/l		100 mg/l		330 mg/l		1000 mg/l		3300 mg/l	
	M	F	M	F	M	F	M	F	M	F	M	F
Water consumption, week 13 (g/day)	4.1	6.0	5.0	5.8	4.6	6.3	4.4	5.6	4.2	5.1	3.1*	3.4*
Haemoglobin (g/dl)	17.6	18.6	17.6	18.2	17.5	17.8	17.7	18.4	17.1*	17.3*	15.9*	18.0
Erythrocytes (10^6/µl)	10.5	9.6	10.5	10.3	10.3	10.2	10.5	10.4	10.2	10.0	9.5*	10.5*
Reticulocytes (10^6/µl)	0.23	0.20	0.26	0.18	0.19	0.17	0.22	0.19	0.24	0.18	0.25	0.24
Pancreas acinar cell degeneration (n = 10)	0	0	0	0	0	0	0	0	2	0	10*	9*
Pancreas fatty change (n = 10)	0	0	0	0	0	0	0	1	2	0	9*	4
Hepatocyte vacuolation (n = 10)	0	0	0	7*	0	10*	0	7*	0	9*	0	10*
Epididymis weight (g)	0.053	—	0.049	—	—	—	0.048	—	—	—	0.059*	—
Abnormal sperm (%)	1.4	—	1.8	—	—	—	1.6	—	—	—	1.7	—

From NTP (1998)
F, female; M, male; * $P < 0.05$

and pancreas, liver and kidneys from all groups were examined histopathologically. An extensive examination of sperm morphology, numbers and motility was performed on all male animals from the control, 33, 330 and 3300 mg/l groups. Estrous cycling was examined in all females.

One male from the 330 mg/l group died during the study. Mice in the top-dose groups had significant reductions (20–40%) in water consumption. Erythrocyte parameters indicative of mild anaemia were present in males at and above 1000 mg/l (Table 8). Pancreatic acinar cell alterations together with pancreatic fatty change were present in both sexes at 3300 mg/l. Vacuolation of hepatocytes (primarily centrilobular) was significantly increased in all female groups, but not in males (Table 8). The severity of renal tubule vacuolation was increased in males at and above 1000 mg/l. Epididymal weights were increased at 3300 mg/l, but there were no adverse effects on measured sperm parameters (Table 8).

No NOAEL could be determined because of the high incidences of hepatocyte vacuolation in all treated female groups (NTP, 1998).

Groups of F344/N rats (10 of each sex per dose) received 1-chloro-2-propanol (batch No. B15) in drinking-water at 0, 100, 330, 1000, 3300 or 10 000 mg/l for 14 days. All animals were necropsied and major organs weighed. An extensive range of tissues from control and top-dose animals and pancreas, spleen, bone marrow and uterus from selected groups were examined histopathologically. Achieved doses were not reported.

Two females from the 10 000 mg/l group died. Rats in the top-dose groups lost weight and had an 80% reduction in water consumption. At 3300 mg/l, body weight gain was reduced by approximately 30%, with water consumption reduced by 40–50%. Haematopoiesis was present in the spleen of the 3300 mg/l groups. Pancreatic acinar cell alterations together with pancreatic degeneration and bone marrow atrophy were present in both sexes at and above 3300 mg/l and in females at 1000 mg/l.

A NOAEL was not determined in females, as the pancreas was not examined at doses below 1000 mg/l (approximately 200 mg/kg bw per day, based on the values for the 14-week study) (NTP, 1998).

Groups of F344/N rats (10 of each sex per dose) received 1-chloro-2-propanol (batch No. B15) in drinking-water at 0, 33, 100, 330, 1000 or 3300 mg/l for 14 weeks. Achieved doses were reported

Table 9. Findings in a 14-week rat study with propylene chlorohydrin in drinking-water

	0 mg/l		33 mg/l		100 mg/l		330 mg/l		1000 mg/l		3300 mg/l	
	M	F	M	F	M	F	M	F	M	F	M[a]	F
Body weight gain (g)	267	108	257	106	254	109	260	104	258	100	196*	82*
Water consumption, week 13 (g/day)	22	18	21	17	23	17	22	19	20	17	14*	9*
Haemoglobin (g/dl)	16.4	16.9	16.7	16.3*	16.5	16.7	16.7	16.7	16.4	15.9*	15.7*	16.0*
Erythrocytes (10^6/µl)	8.7	8.3	8.9	8.1	8.7	8.3	9.1	8.3	8.9	7.9*	8.6	7.6*
Reticulocytes (10^6/µl)	0.26	0.15	0.22	0.20*	0.21	0.19	0.22	0.18	0.22	0.18	0.27	0.33*
Pancreas acinar cell degeneration (n = 10)	0	0	0	0	0	0	0	0	10*	10*	9*	10*
Pancreas fatty change (n = 10)	0	0	0	0	0	0	0	1	10*	10*	9*	10*
Hepatocyte vacuolation (n = 10)	0	—	1	—	6*	—	2	—	7*	—	10*	—
Cauda epididymis weight (g)	0.15	—	0.14	—	—	—	0.14	—	—	—	0.12*	—
Epididymis weight (g)	0.47	—	0.45	—	—	—	0.47	—	—	—	0.42*	—
Abnormal sperm (%)	0.78	—	0.90	—	—	—	0.86	—	—	—	1.5*	—

From NTP (1998)
F, female; M, male; * $P < 0.05$
[a] $n = 9$.

to be 0, 5, 10, 35, 100 and 220 mg/kg bw per day. All animals were necropsied and major organs weighed. Blood samples for clinical chemistry were taken on days 3, 15 and 45 and at study termination. Urinalysis was performed on day 14 and at study termination. A sample for haematology was taken at study termination. An extensive range of tissues from control and top-dose animals and pancreas (both sexes) and liver (males) from all groups were examined histopathologically. An extensive examination of sperm morphology, numbers and motility was performed on all male animals from the control, 33, 330 and 3300 mg/l groups. Estrous cycling was examined in all females.

There were no deaths during the study and no notable effects on estrous cycling or clinical chemistry. Rats in the top-dose groups had significant reductions in body weight gain (20–25%) and in water consumption (40–50%). Urinalysis results were consistent with the reduced water intake, showing a reduction in volume and an increase in specific gravity at the top dose level. Erythrocyte parameters indicative of mild anaemia were present in both sexes at 3300 mg/l and in females dosed at 1000 mg/l (Table 9). Pancreatic acinar cell alterations together with pancreatic fatty change were present in both sexes at and above 1000 mg/l. Vacuolation of hepatocytes (primarily centrilobular) was significantly increased in males from the 100, 1000 and 3300 mg/l groups, but not at 330 mg/l (Table 9); the finding at 100 mg/l is not considered as adverse, as it was not seen at 330 mg/l, and the NTP (1998) report indicated that the change is considered to be of minimal biological significance. Epididymal weights were reduced and abnormal sperm numbers were increased at 3300 mg/l (Table 9).

A NOAEL of 330 mg/l (equal to 35 mg/kg bw per day) was determined, based on the range of findings at 1000 mg/l (NTP, 1998).

Long-term studies of toxicity and carcinogenicity

Groups of B6C3F1 mice (50 of each sex per dose) received 1-chloro-2-propanol (batch No. B15) in drinking-water at 0, 250, 500 or 1000 mg/l for 2 years. All animals were necropsied, and an extensive range of tissues from all animals were examined histopathologically. Achieved doses were 0, 45, 75 and 150 mg/kg bw per day in males and 0, 25, 50 and 100 mg/kg bw per day in females.

Survival was good (> 60% at termination) and similar across treated and control groups. There were no effects on body weight, water consumption or histopathology findings. Tumour incidences and patterns were similar in treated and control groups.

The NOAEL for general toxicity and carcinogenicity was 1000 ppm (equal to 100 mg/kg bw per day), the highest dose tested (NTP, 1998).

Groups of F344/N rats (50 of each sex per dose) received 1-chloro-2-propanol (batch No. B15) in drinking-water at 0, 150, 325 or 650 mg/l for 2 years. All animals were necropsied, and an extensive range of tissues from all animals were examined histopathologically. Achieved doses were 0, 8, 17 or 34 mg/kg bw per day.

Survival was acceptable in treated groups (> 45% in males and > 60% in females at termination) and similar across treated and control groups. There were no effects on body weight, water consumption or histopathology findings. Tumour incidences and patterns were similar in treated and control groups.

The NOAEL for general toxicity and carcinogenicity was 650 ppm (equal to 34 mg/kg bw per day), the highest dose tested (NTP, 1998).

Genotoxicity

Testing of the genotoxicity of propylene chlorohydrin has been performed in a range of assays. The overall database is considered adequate to conclude that propylene chlorohydrin is an in vitro genotoxin. The response in the chromosomal aberration assay in the presence of S9 was very high, with 75% or more aberrant cells compared with 4% in controls. The weight of evidence is that propylene chlorohydrin is not genotoxic in vivo.

Key data on the genotoxicity of propylene chlorohydrin are summarized in Table 10.

Reproductive and developmental toxicity

In a continuous-breeding protocol, groups of Sprague-Dawley rats (VAF Crl:CD BR) received 1-chloro-2-propanol (batch No. B15) in the drinking-water at 0, 300, 650 or 1300 mg/l. There were 40 control pairs and 20 treated pairs per dose level. Exposure was for 112 days for both sexes, plus 21 days in females for delivery of the final litter. Parameters examined included pregnancy index, litters per pair, litter size, pup viability and pup body weight. Pups from the final litters (20 of each sex) from the control and 1300 mg/l groups were allowed to mature and mated to produce the F_2 litters. At necropsy of the final litter (F_1 parents), epididymal sperm data were collected from control and top-dose animals, and reproductive organs were weighed and examined histopathologically.

Maternal body weights were reduced at 650 and 1300 mg/l. There were no significant or consistent changes to reproductive parameters over the five litters produced. The only effects seen in the final F_1 litters were reduced body weights of pups at 650 (~10%) and 1300 mg/l (~20%). In the mating for the F_2 litter (control and 1300 mg/l only), the mean weight of dams at delivery was reduced (~20%), and there was an increase in abnormal epididymal sperm (2.4% versus 0.8% in controls) in male parents; the estrous cycle was slightly extended (4.6 days versus 4.2 days in controls), but this was not statistically significant. These effects had no impact on reproductive outcome, as there were no effects on any reproductive parameters or pup viability.

The NOAEL for reproductive toxicity was 1300 ppm (equal to 130 mg/kg bw per day), the highest dose tested. The NOAELs for offspring and parental toxicity were 300 ppm (equal to 30 mg/kg bw per day), based on deficits in body weight at 650 ppm (NTP, 1998).

Groups of female rats (five per group) received propylene chlorohydrin by gavage at a dose of 0, 8, 20, 50 or 125 mg/kg bw per day on days 6–15 of gestation. Fetuses were examined only for gross external abnormalities.

Maternal body weight gain was reduced at the top dose level of 125 mg/kg bw per day. There were no treatment-related increases in external findings and no effects on viable fetal numbers.

Table 10. Results of studies of genotoxicity with propylene chlorohydrin (1-chloro-2-propanol)

Vehicle	End-point	Test object	Concentration	Purity (%); supplier	Result	Reference
In vitro						
—	Reverse mutation	*Salmonella typhimurium* strains TA97, TA98, TA100, TA1535, TA1537	100–10 000 µg/plate, with and without S9 mix	Not specified; Radian Corp., Austin, TX, USA	Positive with TA1535 (±S9), equivocal with TA100; negative with other strains	NTP (1998)
—	Chromosomal aberrations	Chinese hamster ovary cells	3000, 4000 or 5000 µg/ml, with and without S9 mix	Not specified; Radian Corp., Austin, TX, USA	Positive (±S9)	NTP (1998)
—	Sister chromatid exchanges	Chinese hamster ovary cells	167–4000 µl/plate without S9 mix; 167–1700 µl/plate with S9 mix	Not specified; Radian Corp., Austin, TX, USA	Positive (±S9)	NTP (1998)
Acetone	Reverse mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537	Up to 120 µmol/plate without S9	99; Merck-Schuschardt	Positive with TA100 and TA1535; less potent (~10-fold) than propylene oxide	Pfeiffer & Dunkelberg (1980)
In vivo						
—	Sex-linked recessive mutation	*Drosophila melanogaster* (feed)	200 mg/kg for 3 days	Not specified; Radian Corp., Austin, TX, USA	Negative	NTP (1998)
Saline	Sex-linked recessive mutation	*D. melanogaster* (injection into abdomen)	1000 mg/l	Not specified; Radian Corp., Austin, TX, USA	Positive	NTP (1998)
Saline	Reciprocal chromosome translocations	*D. melanogaster* (injection into abdomen)	1000 mg/l	Not specified; Radian Corp., Austin, TX, USA	Negative	NTP (1998)
Drinking-water	Micronucleus assay	B6C3F1 mouse peripheral blood	0, 33, 100, 330, 1000, 3300 mg/l for 14 weeks	Not specified; Radian Corp., Austin, TX, USA	Negative	NTP (1998)

This study is inadequate, in terms of group size and extent of investigations, to permit identification of a NOAEL for developmental toxicity (Phillips, 1980).

(d) Studies on propylene bromohydrin

Propylene bromohydrin (1-bromo-2-propanol; 2-bromo-1-propanol) is a plant metabolite formed following the use of propylene oxide. No in vivo toxicity data were available for evaluation. Genotoxicity data show that propylene bromohydrin is genotoxic in vitro. Comparative data indicate that in some bacterial mutagenicity tests, the bromopropanol derivatives are more potent mutagens than the equivalent chloro- compounds (NTP, 1985).

(e) Studies on propylene glycol

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established an acceptable daily intake (ADI) of 0–25 mg/kg bw per day for propylene glycol (1,2-propanediol), last evaluated in 2002 (FAO/WHO, 2002).

3. Observations in humans

3.1 Workplace monitoring

(a) Propylene oxide

Haemoglobin adducts (hydroxypropylvaline) were increased significantly in a starch alkylation factory, from 20 pmol/g haemoglobin in controls to 230–3500 pmol/g haemoglobin in exposed workers. In a Chinese factory, a group of eight workers exposed to propylene oxide had hydroxypropylvaline levels of 0.13–4.9 nmol/g haemoglobin compared with a control level of less than 0.01 nmol/g haemoglobin (Czene et al., 2002). 1-Hydroxypropyladenine was reported in the leukocytes of a group of workers at a Chinese propylene oxide production plant (Czene et al., 2002). Epidemiological studies of workers exposed to propylene oxide as well as other chemicals have been inconclusive. A number of cohort studies have been performed covering workers exposed to ethylene oxide and propylene oxide. IARC (1994, 2010) concluded that there were limitations to the studies, in particular relating to exposure characterization and correction for confounders such as smoking, and that further epidemiological investigations of propylene oxide–exposed cohorts should be performed.

(b) Propylene chlorohydrin

An epidemiological study, published in 1990, of workers at a plant producing both ethylene chlorohydrin and propylene chlorohydrin reported an excess of mortality due to pancreatic cancer (odds ratio 4.2) and leukaemia (odds ratio 7.5) in operators working in the chlorohydrin unit for over 2 years. Nine of the 10 decedents had worked in the plant between 1935 and 1945. There were significant trends with duration of employment in the chlorohydrin plant and both cancers. Although no data are available on exposures to chlorohydrins or related chemicals, the authors concluded that the pancreatic cancers and leukaemias were associated with the production of ethylene chlorohydrin and/or propylene chlorohydrin (Greenberg, Ott & Shore, 1990). A second study at the plant confirmed increased risks for pancreatic cancer and leukaemia and identified increased risks with increasing duration of employment for total cancers and all lymphatic and haematopoietic cancers. The authors suggested that exposure to high concentrations of ethylene dichloride, possibly with other chlorinated hydrocarbons, was the most likely causative agent (Benson & Teta, 1993).

Comments

Biochemical aspects

There are no reliable in vivo data on the kinetics or biotransformation of propylene oxide. By analogy with ethylene oxide, it is likely that propylene oxide is rapidly and extensively absorbed via the inhalation route. Oral exposure to propylene oxide is likely to result in hydrolysis to propylene glycol in the stomach. In vitro work has shown that propylene oxide hydrolyses significantly more rapidly in human synthetic gastric juice (pH 1.46; half-life ~2 minutes) than in rat synthetic gastric juice (pH 4.8; half-life > 2 hours). Absorbed propylene oxide is likely to be hydrolysed to propylene glycol by epoxide hydrolase or bind to non-protein sulfhydryl groups, such as glutathione. There are no data that permit comparison of systemic exposures to propylene oxide by the inhalation and oral

routes. It is expected that inhalation exposures to propylene oxide will result in greater systemic levels than equivalent oral exposures when account is taken of the likely hydrolysis rates in the human stomach combined with kinetic data on propylene oxide levels in blood following inhalation exposure and a physiologically based pharmacokinetic model for inhalation exposures to propylene oxide.

For the purposes of this assessment, a simplistic conversion between inhalation exposures and oral dosing has been performed. For rats, this conversion assumed standard breathing rates and volumes, a body weight of 250 g and 100% absorption via each exposure route. The conversion resulted in an atmospheric concentration of 100 ppm (240 mg/m^3) inhaled for 6 hours/day, 5 days/week, being approximately equivalent to an oral dose of 40 mg/kg bw per day in rats. The corresponding value for mice is considered to be approximately double that for rats, giving 100 ppm equivalent to 80 mg/kg bw per day. These are likely to be conservative estimates for systemic propylene oxide exposures via the oral route.

Toxicological data

The acute toxicity of propylene oxide has been investigated orally (mouse and rat LD_{50}s 300–1000 mg/kg bw), dermally (rabbit LD_{50}s 950–1250 mg/kg bw) and by inhalation (mouse and rat LC_{50}s 1.0–9.5 mg/l). Propylene oxide is an irritant to skin, respiratory tract and eyes. There are no data on its sensitizing potential.

Short-term studies of toxicity with propylene oxide have been performed in mice and rats, mainly via the inhalation route, in which no systemic effects other than body weight deficits were evident. No effects on the nasal cavity were reported in rats or mice exposed for 13 weeks (6 hours/day, 5 days/week) at up to 500 ppm. In a gavage study in rats dosed 18 times in 24 days, reduced body weight gain, gastric irritation and hepatotoxicity were reported at 300 mg/kg bw per day, with a NOAEL of 200 mg/kg bw per day.

In a chronic toxicity and carcinogenicity study in mice exposed via inhalation at 200 or 400 ppm for 6 hours/day, 5 days/week, survival was reduced at both concentrations. Body weights were significantly lower in the 400 ppm groups during the second half of the study. Inflammation of the nasal epithelia was seen in all treated groups. Low incidences of squamous cell carcinoma and adenocarcinoma of the nasal epithelia were present in high-dose animals. There was also an increase in haemangiosarcoma and haemangioma of the vascular plexus below the nasal epithelium. An increase in mammary gland adenocarcinoma was seen in females, which was statistically significant in the high-dose group when corrected for survival; the incidences are within the historical control range and considered to be not clearly treatment related. A NOAEC for site of contact toxicity cannot be derived for this study due to the inflammation of the nasal epithelia seen at both concentrations. The NOAEC for carcinogenicity was 200 ppm (equivalent to approximately 160 mg/kg bw per day orally), based on the nasal tumours seen at 400 ppm (equivalent to approximately 320 mg/kg bw per day orally). The NOAEC for systemic toxicity was 200 ppm (equivalent to approximately 160 mg/kg bw per day orally), based on reduced body weight gain at 400 ppm (equivalent to approximately 320 mg/kg bw per day orally).

In a published 150-week study, female rats were exposed to propylene oxide by gavage twice a week at 15 or 60 mg/kg bw per administration (equal to 4.3 or 17 mg/kg bw per day). The extent of the tissues examined and level of reporting are less than those carried out in a normal regulatory study, with minimal or no reporting of body weights, clinical signs or non-neoplastic lesions. Within the limitations of the investigative procedure, the only organ with an increased incidence of non-neoplastic lesions (hyperkeratosis) or tumours was the stomach/forestomach (data not presented separately). The incidence of squamous cell carcinoma in the stomach/forestomach showed a clear dose–response relationship. The lowest dose level gave a slight increase in squamous cell carcinoma of the stomach/forestomach. The NOAEL for carcinogenicity was less than 4.3 mg/kg bw per day.

The study did not demonstrate a NOAEL for chronic toxicity because of the presence of hyperkeratosis at 4.3 mg/kg bw per day, the lowest dose tested.

In a 28-month inhalation study in rats, survival was reduced in the 300 ppm groups and in 100 ppm females at the end of the study (after week 115). Body weights were reduced in the 300 ppm groups. Increases in relative liver weights (10–15%) were statistically significant at 300 ppm in males sacrificed at 24 and 28 months and in females sacrificed at 24 months. Local effects on the basal mucosa, nasal turbinates and olfactory epithelium were seen at 300 ppm and occasionally at 100 ppm from 12 months onwards. Non-neoplastic findings were seen in the heart, liver, lung and kidneys at 300 ppm; the effects at 100 and 30 ppm are unclear because of the limited number of tissues examined. There were no increases in tumour incidence in the nose or respiratory tract. Increased incidences of mammary gland fibroadenomas and thyroid tumours (follicular cell adenoma and parafollicular cell adenoma) were recorded in the 300 ppm groups. The incidences of multiple mammary gland tumours were increased in all treated female groups but were reported to be within the historical control range. A NOAEC for systemic effects was 100 ppm (equivalent to approximately 40 mg/kg bw per day orally), based on body weight gain reductions at 300 ppm (equivalent to approximately 120 mg/kg bw per day orally). The increased mortality at 100 ppm at week 115 is not considered relevant, as this is beyond the normal lifespan of laboratory rats.

In a second chronic inhalation study, rats were exposed to propylene oxide at 0, 200 or 400 ppm for 6 hours/day, 5 days/week, for 2 years. Body weights were slightly lower (< 10%) in the 400 ppm groups than in controls. Inflammation of the nasal cavity was increased at 400 ppm and in males at 200 ppm. Tumours of the nasal cavity (papillary adenoma) were increased in both sexes at 400 ppm, outside the historical control range. Other tumours showing increased incidences were mammary gland, uterus and thyroid tumours in females. The uterine stromal sarcoma incidences were above the historical control range at both concentrations of propylene oxide, but did not exhibit a dose–response relationship. The thyroid gland C-cell tumours were at the upper end of the historical control range, and as there was no related increase in hyperplasia, the relationship to propylene oxide is considered equivocal. The mammary gland tumours were not increased statistically significantly and were within the historical control range, but are consistent with results in other studies, and their relationship to propylene oxide is equivocal. The NOAEC for tumours was 200 ppm (equivalent to approximately 80 mg/kg bw per day orally), based on the increase in papillary adenomas of the nasal cavity at 400 ppm (equivalent to approximately 160 mg/kg bw per day orally). The NOAEC for chronic site of contact toxicity was less than 200 ppm (equivalent to approximately 80 mg/kg bw per day orally), based on nasal cavity inflammation. For systemic toxicity, the NOAEC was 200 ppm (equivalent to approximately 80 mg/kg bw per day orally), based on reduced body weight gain at 400 ppm (equivalent to approximately 160 mg/kg bw per day orally).

Evidence of carcinogenicity was seen in long-term studies of toxicity and carcinogenicity with propylene oxide in rats via both oral (stomach/forestomach) and inhalation routes (nasal cavity and mammary tumours) and in mice via inhalation (nasal cavity and mammary tumours). The relevance of these tumours to human exposures to relatively low levels of propylene oxide via the diet is equivocal. In vitro work has shown that propylene oxide hydrolyses significantly more rapidly in human synthetic gastric juice than in rat synthetic gastric juice. This indicates that the stomach tumours seen in the rat gavage study might be associated with a much more prolonged exposure to propylene oxide than would occur in humans.

Similarly, for the nasal cavity tumours seen in the inhalation studies with rats and mice, these could be associated with chronic irritation of the epithelial cells and depletion of sulfhydryl groups and not relevant to oral exposures. However, there have been no specific mechanistic investigations to demonstrate that site of contact mutagenic effects do not occur. A threshold concentration for nasal tumours in chronic studies appears to be 300 ppm (720 mg/m^3), which is consistent with data on non-protein sulfhydryl group depletion in nasal mucosa.

In mice and rats exposed to propylene oxide by inhalation, increases in mammary tumours were noted, but these were reported to be within the historical control ranges.

The Meeting concluded that there was no convincing evidence that propylene oxide caused systemic tumorigenicity in mice and rats.

The potential genotoxicity of propylene oxide has been investigated in an adequate battery of tests in vitro and in vivo. Positive results were seen in a range of in vitro assays. In vivo assays (for micronuclei and dominant lethal mutations) using oral administration were negative; positive results were seen following high-dose intraperitoneal administration in mice and a high-concentration inhalation study in fruit flies. There are no in vivo data from tissues directly exposed to propylene oxide rather than its metabolites. Propylene oxide produces DNA adducts (primarily N^7G, plus N^3A, N^3C and N^1A) in respiratory mucosa and liver of exposed rats, and 1-hydroxypropyladenine was reported in the leukocytes of a group of propylene oxide production plant workers.

The Meeting concluded that propylene oxide is genotoxic in vitro but is unlikely to be genotoxic via the oral route due to hydrolysis to propylene glycol in the stomach.

The Meeting concluded that propylene oxide is carcinogenic to experimental animals at the site of initial contact, but because of the likely rapid hydrolysis to propylene glycol in the human stomach and negative genotoxicity in vivo via oral administration, it is unlikely to be carcinogenic to humans following exposure via the oral route to propylene oxide residues in the diet.

No oral studies of reproductive toxicity or developmental toxicity are available. In a rat reproductive toxicity study using inhalation exposure, there were no effects reported on mating performance, fertility, litter size, pup survival or development at the highest concentration tested (300 ppm, 6 hours/day, 5 days/week). Reduced body weight gain was seen in parental animals and pups at 300 ppm. The NOAEC for reproductive toxicity was 300 ppm (equivalent to approximately 120 mg/kg bw per day orally), the highest dose tested. The NOAEC for parental and pup toxicity was 100 ppm (equivalent to approximately 40 mg/kg bw per day orally), based on reduced body weight gain at 300 ppm (equivalent to approximately 120 mg/kg bw per day orally).

The Meeting concluded that propylene oxide does not adversely affect reproduction via the inhalation route at exposure concentrations producing parental toxicity.

In a well-reported developmental toxicity study, rats were exposed to propylene oxide at 0, 100, 300 or 500 ppm for 6 hours/day on days 6–15 of gestation. Maternal body weight gain was reduced at 500 ppm. There was no increase in malformations, and the NOAEC for teratogenicity was 500 ppm (equivalent to approximately 260 mg/kg bw per day orally).[1] There were no effects on litter size, post-implantation losses, fetal viability or litter size. The only significant developmental finding was an increase in accessory cervical ribs at 500 ppm. The NOAECs for maternal and developmental effects were both 300 ppm (equivalent to approximately 160 mg/kg bw per day orally). In a limited developmental toxicity study, rats were exposed by inhalation to a single concentration of propylene oxide (500 ppm) for 7 hours/day during various phases of gestation. Body weight gain was reduced in treated animals, whereas kidney, liver, lung and spleen weights were increased. There were decreases reported in numbers of corpora lutea and implantation sites and live fetus weights, length and numbers. The only visceral, skeletal or external alterations were increased incidences of wavy ribs and reduced ossification of the ribs and vertebrae in the exposed groups. The single air concentration tested (500 ppm; equivalent to approximately 200 mg/kg bw per day orally) is a NOAEC for teratogenicity and a LOAEC for maternal and developmental toxicity. In an almost identical study in rabbits, there were reductions reported in maternal body weight gain, histopathological changes in a number of organs and increases in resorptions and minor skeletal abnormalities. There were no reported increases in malformations. The single concentration tested (500 ppm; equivalent

[1] A different conversion rate was used, as exposures occurred every day as opposed to 5 days/week.

to approximately 75 mg/kg bw per day orally) is reported to be a NOAEC for teratogenicity and a LOAEC for maternal and developmental toxicity in rabbits.

The Meeting concluded that propylene oxide produced developmental toxicity via the inhalation route, but the available evidence indicated that it was not teratogenic.

Hydroxypropylvaline adducts of haemoglobin have been detected in workers in industrial facilities using or producing propylene oxide. 1-Hydroxypropyladenine was reported in the leukocytes of a group of propylene oxide production plant workers. Epidemiological studies of workers exposed to propylene oxide as well as other chemicals have been inconclusive.

Biochemical and toxicological data on propylene chlorohydrin

Propylene chlorohydrin (1-chloro-2-propanol, 2-chloro-1-propanol) is a plant metabolite formed following the use of propylene oxide. Data have been generated on a 3:1 mixture of 1-chloro-2-propanol and 2-chloro-1-propanol.

Biochemical aspects

Limited, qualitative data indicate that propylene chlorohydrin is absorbed following oral administration, conjugated to glucuronic acid or glutathione and excreted in the urine.

Toxicological data

The acute toxicity of propylene chlorohydrin has been investigated via the oral route (rat LD_{50} 200–250 mg/kg bw), the dermal route (rabbit LD_{50} 500 mg/kg bw) and inhalation (rat $LC_{50} > 3.8$ mg/l). Propylene chlorohydrin is not irritating to rabbit skin but is a severe eye irritant. There are no data on its skin sensitizing potential.

In a 14-day drinking-water study in mice, reductions in body weight were seen at the top dose level (10 000 mg/l). Alterations in pancreatic acinar cells and pancreatic degeneration and hepatocyte vacuolation were reported at 3300 mg/l and above. The NOAEL was 330 mg/l (equivalent to 33 mg/kg bw per day), based on hepatocyte vacuolation at 1000 mg/l (equivalent to 100 mg/kg bw per day). In a subsequent 14-week study, findings were similar (including pancreatic acinar cell degeneration and fatty change of the pancreas), but it was not possible to identify a NOAEL due to hepatocyte vacuolation at the lowest dose tested, 33 mg/l (equal to 7 mg/kg bw per day).

In a 14-day drinking-water study in rats, reduced body weight was seen at high dose levels. Indications of red cell effects (splenic haematopoiesis, bone marrow atrophy) were present at 3300 mg/l and above, and pancreatic degeneration/acinar cell changes were seen at 3300 mg/l and above in males and at 1000 mg/l and above in females. A NOAEL could not be determined because of the limited investigations at dose levels below 1000 mg/l (equal to 100 mg/kg bw per day). In an equivalent 14-week study, body weight, erythrocyte, pancreas and liver effects were seen at 1000 mg/l, with a NOAEL of 330 mg/l (equal to 35 mg/kg bw per day).

Chronic toxicity and carcinogenicity studies have been performed in mice and rats exposed to propylene chlorohydrin in the drinking-water for 2 years. In both of the studies, there were no indications of carcinogenicity or general toxicity, including of the pancreas and liver. Haematological and clinical chemistry examinations were not performed. The NOAELs were the highest concentrations tested, 1000 mg/l (equal to 100 mg/kg bw per day) in mice and 650 mg/l (equal to 34 mg/kg bw per day) in rats.

The potential genotoxicity of propylene chlorohydrin has been investigated in an adequate battery of tests in vitro and in vivo. Positive results were seen in a range of in vitro assays. Negative results were seen in vivo with oral administration, although a mutation assay in *Drosophila* using injection administration was positive.

The Meeting concluded that propylene chlorohydrin is genotoxic in vitro but unlikely to be genotoxic in vivo.

Taking note of the absence of genotoxicity in vivo in mammals and the absence of carcinogenicity in rats and mice, the Meeting concluded that propylene chlorohydrin is unlikely to be carcinogenic to humans.

In a continuous breeding reproductive toxicity study, rats were exposed to propylene chlorohydrin in drinking-water over two generations. Reduced body weight gain was seen in dams and pups at 650 mg/l. There were no adverse effects on reproduction or pup viability at any dose level. An increase in numbers of abnormal sperm and slightly extended estrus were reported in parental animals at 1300 mg/l, but these were without any reproductive consequence and are considered not to be adverse. The reproductive NOAEL was 1300 mg/l (equal to 130 mg/kg bw per day), the highest dose tested. The NOAEL for parental toxicity was 300 mg/l (equal to 30 mg/kg bw per day), based on reduced body weights at 650 mg/l (equal to 65 mg/kg bw per day). The NOAEL for offspring toxicity was 300 mg/l (equal to 30 mg/kg bw per day), based on reduced body weight gain at 650 mg/l (equal to 65 mg/kg bw per day).

The Meeting concluded that propylene chlorohydrin is not toxic to reproduction.

In a limited developmental toxicity study, propylene chlorohydrin was administered to five pregnant rats per group. Fetuses were examined only for gross external abnormalities. Maternal body weight gain was reduced at the top dose level of 125 mg/kg bw per day. There were no treatment-related increases in external findings and no effects on viable fetal numbers. This study is inadequate, with respect to group size and extent of investigations, to permit identification of a NOAEL for developmental toxicity.

Epidemiological studies of workers in plants producing propylene chlorohydrin and other chlorinated hydrocarbons identified an excess of mortality due to pancreatic cancer, leukaemia, and all lymphatic and haematopoietic cancers. The involvement, if any, of propylene chlorohydrin in these effects is unclear.

Toxicological data on propylene bromohydrin

Propylene bromohydrin (1-bromo-2-propanol; 2-bromo-1-propanol) is a plant metabolite formed following the use of propylene oxide. No in vivo toxicity data were available for evaluation. Genotoxicity data show that propylene bromohydrin is genotoxic in vitro. Comparative data indicate that in some bacterial mutagenicity tests, the bromopropanol derivatives are more potent mutagens than the equivalent chloro- compounds.

Toxicological data on propylene glycol

Propylene glycol (1,2-propanediol) is a plant metabolite formed following the use of propylene oxide. It is also an approved food additive (e.g. E1520). It was reviewed by JECFA in 2002, when an ADI of 0–25 mg/kg bw was derived.

The Meeting concluded that the existing database on propylene oxide was adequate to characterize the potential hazards to fetuses, infants and children by the inhalation route. Taking account of the likely hydrolysis to propylene glycol following oral exposure, the inhalation studies are considered to provide adequate reassurance for potential risks to fetuses, infants and children via the oral route.

The Meeting concluded that the existing database on propylene chlorohydrin was adequate to characterize the potential hazards to infants and children, but not to fetuses.

Toxicological evaluation

Propylene oxide

The Meeting established an ADI of 0–0.04 mg/kg bw derived from the NOAEC for systemic effects (reduced body weight gain) in the chronic inhalation studies in rats of 100 ppm (equivalent to approximately 40 mg/kg bw per day orally), supported by the NOAEC of 100 ppm (equivalent to approximately 40 mg/kg bw per day orally) for offspring and parental toxicity (reduced body weight gain) in the reproductive toxicity study in rats. Kinetic and metabolic data indicate that there is likely to be greater systemic exposure to propylene oxide following inhalation exposures relative to equivalent oral exposures; thus, the extrapolation is likely to be conservative. A safety factor of 1000 was applied. An additional factor of 10 was applied to the default safety factor of 100 to address the limitations in the database. The 150-week oral study in rats was not used in the establishment of the ADI, as there was limited investigation of non-neoplastic systemic effects and the critical findings reported were local effects in the rat stomach that are considered not relevant to human exposures to propylene oxide residues in the diet.

The Meeting established an acute reference dose (ARfD) of 0.04 mg/kg bw on the same basis as the ADI. The Meeting concluded that there was inadequate information to support the derivation of a value based on specific acute effects.

Propylene chlorohydrin

The Meeting could not establish an ADI or ARfD for propylene chlorohydrin because of the absence of any reliable data to characterize the hazards to fetuses. The chemical properties and toxicity profile of propylene chlorohydrin are different from those of propylene oxide, and it is not possible to read across between the two compounds.

Propylene bromohydrin

The Meeting could not establish an ADI or ARfD for propylene bromohydrin because of the absence of any in vivo data. The chemical properties of propylene bromohydrin are different from those of propylene oxide, and it is not possible to read across between the two compounds.

Levels relevant to risk assessment of propylene oxide

Species	Study	Effect	NOAEL/C	LOAEL/C
Mouse	Two-year study of toxicity and carcinogenicity[a]	Systemic toxicity	200 ppm (~160 mg/kg bw per day orally)[b]	400 ppm (~320 mg/kg bw per day orally)[b]
		Carcinogenicity	200 ppm (~160 mg/kg bw per day orally)[b]	400 ppm (~320 mg/kg bw per day orally)[b]
Rat	Twenty-eight-month study of toxicity and carcinogenicity[a]	Systemic toxicity	100 ppm[c] (~40 mg/kg bw per day orally)[b]	300 ppm (~120 mg/kg bw per day orally)[b]
		Carcinogenicity	300 ppm[d] (~120 mg/kg bw per day orally)[b]	—
	Two-year study of toxicity and carcinogenicity[a]	Systemic toxicity	200 ppm (~80 mg/kg bw per day orally)[b]	400 ppm (~160 mg/kg bw per day orally)[b]
		Carcinogenicity	200 ppm (~80 mg/kg bw per day orally)[b]	400 ppm (~160 mg/kg bw per day orally)[b]

Species	Study	Effect	NOAEL/C	LOAEL/C
	Multigeneration study of reproductive toxicity[a]	Reproductive toxicity	300 ppm[d] (~120 mg/kg bw per day orally)[b]	—
		Parental toxicity	100 ppm (~40 mg/kg bw per day orally)[b]	300 ppm (~120 mg/kg bw per day orally)[b]
		Offspring toxicity	100 ppm (~40 mg/kg bw per day orally)[b]	300 ppm (~120 mg/kg bw per day orally)[b]
	Developmental toxicity study[a]	Maternal toxicity	300 ppm (~160 mg/kg bw per day orally)[e]	500 ppm (~260 mg/kg bw per day orally)[e]
		Embryo and fetal toxicity	300 ppm (~160 mg/kg bw per day orally)[e]	500 ppm (~260 mg/kg bw per day orally)[e]
Rabbit	Developmental toxicity study[a]	Maternal toxicity	—	500 ppm[f] (~75 mg/kg bw per day orally)
		Embryo and fetal toxicity	—	500 ppm[f] (~75 mg/kg bw per day orally)

[a] Inhalation exposure.
[b] Conversion of 100 ppm equivalent to 40 mg/kg bw per day in rats, assuming 100 ppm = 240 mg/m^3; 100% absorption; 250 g body weight; standard breathing rates and volumes; exposures for 6 hours/day, 5 days/week. For mice, the corresponding value is double that for rats: 100 ppm equivalent to 80 mg/kg bw per day.
[c] Limited examination.
[d] Highest concentration tested.
[e] Conversion of 100 ppm equivalent to 40 mg/kg bw per day in rats, assuming 100 ppm = 240 mg/m^3; 100% absorption; 250 g body weight; standard breathing rates and volumes; exposures for 6 hours/day on gestation days 6–15.
[f] Lowest concentration tested.

Levels relevant to risk assessment of propylene chlorohydrin

Species	Study	Effect	NOAEL	LOAEL
Mouse	Fourteen-week toxicity study[a]	Toxicity	—	7 mg/kg bw per day[b]
	Two-year study of toxicity and carcinogenicity[a]	Toxicity	100 mg/kg bw per day[c]	—
		Carcinogenicity	100 mg/kg bw per day[c]	—
Rat	Two-year study of toxicity and carcinogenicity[a]	Toxicity	34 mg/kg bw per day[c]	—
		Carcinogenicity	34 mg/kg bw per day[c]	—
	Multigeneration study of reproductive toxicity[a]	Reproductive toxicity	130 mg/kg bw per day[c]	—
		Parental toxicity	30 mg/kg bw per day	65 mg/kg bw per day
		Offspring toxicity	30 mg/kg bw per day	65 mg/kg bw per day

[a] Drinking-water administration.
[b] Lowest dose tested.
[c] Highest dose tested.

Estimate of acceptable daily intake for humans

0–0.04 mg/kg bw for propylene oxide

No ADI could be established for propylene chlorohydrin or propylene bromohydrin.

Estimate of acute reference dose

0.04 mg/kg bw for propylene oxide

No ARfD could be established for propylene chlorohydrin or propylene bromohydrin.

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

- Developmental toxicity data via the oral route for propylene chlorohydrin
- Sufficient information to evaluate the potential toxicity of propylene bromohydrin residues in the diet

For further information, see Environmental Health Criteria 240 (FAO/WHO, 2009).

Critical end-points for setting guidance values for exposure to propylene oxide

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	No data
Dermal absorption (human skin in vitro)	No data
Distribution	No data
Potential for accumulation	Unlikely
Rate and extent of excretion	No data
Metabolism in animals	Hydrolysed to propylene glycol or conjugated
Toxicologically significant compounds (animals, plants and the environment)	Propylene oxide, propylene chlorohydrin, propylene bromohydrin
Acute toxicity	
Rat, LD_{50}, oral	300–1000 mg/kg bw
Rat, LD_{50}, dermal	950 mg/kg bw
Rat, LC_{50}, inhalation	3.2–3.4 mg/l (4 h, nose only)
Rabbit, dermal irritation	Severe
Rabbit, ocular irritation	Moderate to severe
Dermal sensitization	No data
Short-term studies of toxicity	
Target/critical effect	Body weight gain
Lowest relevant oral NOAEL	200 mg/kg bw per day (rats)
Lowest relevant dermal NOAEL	No data
Lowest relevant inhalation NOAEC	250 ppm (600 mg/m^3) (mice and rats)
Genotoxicity	
	Genotoxic in vitro; unlikely to be genotoxic in humans at dietary exposure levels
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Site of contact irritation (nasal cavity inflammation; stomach hyperkeratosis); systemic toxicity (reduced body weight gain)
Lowest relevant LOAEL	4.3 mg/kg bw per day (lowest dose tested) (rat)
Lowest relevant NOAEC (systemic toxicity)	100 ppm (rat) (~40 mg/kg bw per day oral)
Carcinogenicity	Site of contact tumours (nasal cavity; stomach)
Reproductive toxicity	
Reproduction target/critical effect	None
Lowest relevant reproductive NOAEC	300 ppm (rat) (~120 mg/kg bw per day oral)
Developmental target/critical effect	Accessory cervical ribs (rat)
Lowest relevant developmental NOAEC	300 ppm (rat) (~120 mg/kg bw per day oral)

Neurotoxicity/delayed neurotoxicity	
	No data
Other toxicological studies	
	DNA and haemoglobin adduct formation in rats and humans; depletion of non-protein sulfhydryl groups
Medical data	
	Epidemiological studies of production plant workers inconclusive

Critical end-points for setting guidance values for exposure to propylene chlorohydrin

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	> 11% (limited information)
Dermal absorption (human skin in vitro)	No data
Distribution	No data
Potential for accumulation	Unlikely
Rate and extent of excretion	> 11% (urine, rabbit)
Metabolism in animals	Glucuronide and glutathione conjugates
Toxicologically significant compounds (animals, plants and the environment)	Propylene chlorohydrin
Acute toxicity	
Rat, LD_{50}, oral	200–250 mg/kg bw
Rat, LD_{50}, dermal	500 mg/kg bw
Rat, LC_{50}, inhalation	> 3.8 mg/l (6 h)
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Severe
Dermal sensitization	No data
Short-term studies of toxicity	
Target/critical effect	Liver (hepatocyte vacuolation); pancreas (acinar cell alterations)
Lowest relevant oral NOAEL	35 mg/kg bw per day (rat)
Lowest relevant dermal NOAEL	No data
Lowest relevant inhalation NOAEC	No data
Genotoxicity	
	Genotoxic in vitro; unlikely to be genotoxic in vivo
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	None
Lowest relevant NOAEL	34 mg/kg bw per day (highest dose tested) (rat) 100 mg/kg bw per day (highest dose tested) (mouse)
Carcinogenicity	Not carcinogenic
Reproductive toxicity	
Reproduction target/critical effect	None

Lowest relevant reproductive NOAEL	130 mg/kg bw per day (highest dose tested)
Developmental target/critical effect	Inadequate data
Lowest relevant developmental NOAEC	Inadequate data

Neurotoxicity/delayed neurotoxicity

No data

Other toxicological studies

No data

Medical data

Epidemiological studies of production plant workers inconclusive

Summary for propylene oxide

	Value	Study	Safety factor
ADI	0–0.04 mg/kg bw	Rat chronic inhalation	1000
ARfD	0.04 mg/kg bw	Same basis as ADI; insufficient data to establish a value for specific acute effects	1000

Summary for propylene chlorohydrin

	Value	Study	Safety factor
ADI	None established		
ARfD	None established		

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SAFLUFENACIL

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Explanation

Saflufenacil is the International Organization for Standardization (ISO)–approved name for *N*′-[2-chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-(trifluoromethyl)-3,6-dihydro-1(2H)-pyrimidinyl) benzoyl]-*N*-isopropyl-*N*-methylsulfamide (International Union of Pure and Applied Chemistry), for which the Chemical Abstracts Service number is 372137-35-4. Saflufenacil is a new herbicide from the uracil family of herbicides, acting as a protoporphyrinogen IX oxidase (PPO) inhibitor. Saflufenacil 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 pivotal studies with saflufenacil were certified as complying with good laboratory practice unless stated otherwise.

Evaluation for acceptable daily intake

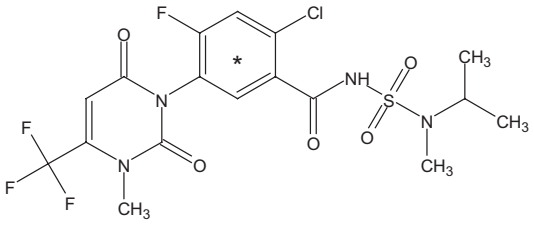
1. Biochemical aspects

The absorption, distribution, metabolism and excretion, as well as the toxicokinetics, of saflufenacil have been investigated in Wistar rats. Summaries of the relevant data are presented below.

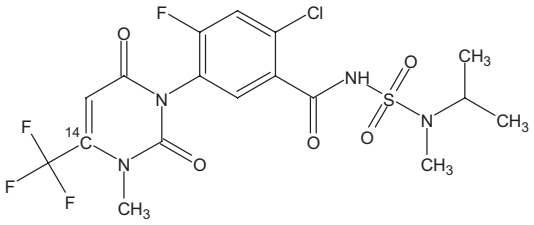
The structure and position of labels on the forms of saflufenacil used in the absorption, distribution, excretion and metabolism studies are shown in Figure 1.

Figure 1. Radiolabelled forms of saflufenacil used in absorption, distribution, metabolism and excretion studies: structure and position of labels

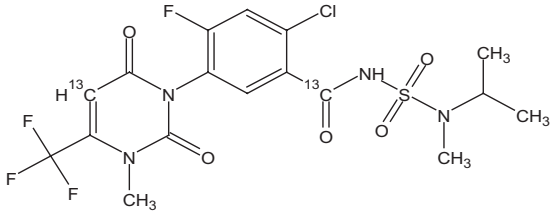
[Phenyl-U-^{14}C]saflufenacil



[Uracil-4-^{14}C]saflufenacil



[Uracil-5-^{13}C, benzamide-carbonyl-^{13}C]saflufenacil



1.1 Absorption, distribution and excretion

The absorption, distribution, excretion and metabolism of orally administered saflufenacil were studied in male and female Wistar rats using (phenyl-U-^{14}C)- and (uracil-4-^{14}C)-labelled saflufenacil. The purity of radioactive saflufenacil used in these studies was greater than 96%. The chemical purity of non-radioactive saflufenacil was greater than 93.9%. In a toxicokinetic study, [phenyl-U-^{14}C]saflufenacil was administered to four Wistar rats of each sex per group via gavage at dose levels of 4, 20 and 100 mg/kg body weight (bw) suspended in an aqueous solution of 0.5% carboxymethylcellulose in plasma kinetics studies and 5 and 100 mg/kg bw for mass balance, tissue distribution and biliary excretion experiments. For plasma kinetics studies, the radioactivity in whole blood and plasma was determined at 1, 2, 4, 8, 24, 48, 72, 96, 120, 144 and 168 hours post-dosing for all three dose levels. Animals were sacrificed at 168 hours post-dosing. In mass balance and tissue distribution studies, urine and faeces were collected at 6 (urine only), 12, 24, 48, 72, 96, 120, 144 and 168 hours post-dosing. Animals were sacrificed at 168 hours after oral dosing, and various tissues were collected and analysed for radioactivity. The metabolism cages were rinsed and analysed for the presence of radioactivity. Expired air was collected from two males per dose for up to 48 hours. A time course tissue distribution study was conducted in three rats of each sex following a single gavage administration of 5 and 100 mg/kg bw. In this study, three males per dose were sacrificed at 1, 24, 48 and 72 hours and at 1, 7, 20 and 34 hours post-dosing at 5 and 100 mg/kg bw, respectively, and three females per dose were sacrificed at 1, 4, 20 and 24 hours and at 1, 7, 20 and 34 hours post-dosing at 5 and 100 mg/kg bw, respectively. In a repeated-dose study, four rats of each sex were administered a daily gavage dose of 100 mg/kg bw of non-radioactive saflufenacil for 14 days followed by a single gavage dose of 100 mg/kg bw of radioactive [phenyl-U-^{14}C]saflufenacil. Urine and faeces were collected at 6 (urine only), 12 (urine only), 24, 48, 72, 96, 120, 144 and 168 hours post-dosing with radioactive compound. Animals were sacrificed at 168 hours after the last dose, and selected tissues were sampled and analysed for the radioactive content. The purpose of the study was to evaluate the influence of multiple dosing on the oral absorption, distribution, excretion and metabolism of [phenyl-U-^{14}C]saflufenacil in rats and to compare these data with results from a single-dose gavage metabolism study. For the biliary excretion studies, bile duct–cannulated rats (four of each sex per dose) received a single gavage saflufenacil dose of 5 or 100 mg/kg bw. To facilitate the elucidation of the structure of metabolites formed, ^{13}C-labelled saflufenacil was added to the saflufenacil preparation for the bile excretion experiment at the high dose level. Bile was collected at 3-hour intervals for 48 hours post-dosing. Bile duct–cannulated animals were sacrificed at 48 hours after the dosing. The experimental procedures and results of these studies are presented in a report by Fabian & Landsiedel (2007a).

The mean total radioactive recoveries in these experiments ranged from 91.9% to 113.1% of the administered dose in both sexes. Less than 2% of the administered dose was recovered as carbon dioxide. Orally administered saflufenacil was rapidly absorbed, and the maximum plasma concentration (C_{max}) was reached within 1 hour of dosing (time to reach maximum concentration, T_{max}) for all dose groups (Table 1). Thereafter, the plasma level of saflufenacil declined rapidly, and only residual radioactivity was detected at 168 hours. A comparable time course of radioactivity was found in blood and plasma of both sexes. A relatively constant blood/plasma ratio between 0.1 and 0.4 was generally found, indicating that major parts of the radioactivity were in plasma and not bound to cellular blood constituents. The area under the plasma concentration–time curve (AUC) values indicated a sex difference, with up to 3-fold higher internal exposures for males than for females, most probably due to a higher clearance of ^{14}C-labelled saflufenacil in female rats. Increasing the dose by a factor of 25 resulted in an increase of the AUC values by a factor of 6.1 in males and 12.4 in females.

After single or repeated oral administration of ^{14}C-labelled saflufenacil, mean total recoveries of radioactivity in the urine and faeces were high for all groups (97–110% of the administered dose at 168 hours; Table 2). In general, the majority of radioactivity was excreted in the urine and faeces within the first 24–48 hours, and excretion was essentially complete within 96 hours. After

Table 1. Toxicokinetic parameters of ^{14}C-labelled saflufenacil in the Wistar rat

Sex	Dose (mg/kg bw)	C_{max} (µg eq/g)	T_{max} (h)	Initial half-life (h)	Terminal half-life (h)	AUC (µg eq·h/g)
Male	100	286.0	1	9.1	33.5	4501.5
	20	98.3	1	8.8	22.6	2131.2
	4	23.9	1	20.9	20.9	741.1
Female	100	258.3	1	4.9	59.2	3056.9
	20	84.8	1	6.5	58.1	754.2
	4	23.0	1	8.1	49.5	246.5

From Fabian & Landsiedel (2007a)
AUC, area under the curve; C_{max}, maximum plasma concentration; eq, equivalents; T_{max}, time to reach C_{max}

196 hours, approximately 26% and 96% of the administered dose were excreted in the urine of male and female rats, respectively, at 5 mg/kg bw. About 52.6% and 86.6% of the administered dose were excreted in the urine of male and female rats, respectively, at 100 mg/kg bw in 196 hours. After 196 hours, approximately 81.2% and 12.8% of the administered dose were excreted in the faeces of male and female rats, respectively, at 5 mg/kg bw. About 43.3% and 9.8% of the administered dose were excreted in the faeces of male and female rats, respectively, at 100 mg/kg bw in 196 hours. The higher excretion of radioactivity in the urine of female rats of the 5 mg/kg bw group may be due to contamination of urine samples with faeces. Radioactivity remaining in tissues at 168 hours was very low and occurred mainly in the carcass (0.06–0.10%), liver (0.03–0.06%), skin (0.02–0.03%) and gut content (0.02–0.03%). The data demonstrated a sex-specific excretion pattern for ^{14}C-labelled saflufenacil, with a higher amount of urinary excretion for females than for males. The sex-dependent excretion was more pronounced at the low dose level than at the high dose level.

Within 1 hour after oral administration of ^{14}C-labelled saflufenacil at 5 or 100 mg/kg bw, the highest radioactivity was found in the stomach and gut contents, carcass, liver and skin (Tables 3, 4 and 5). The radioactivity in the gut content generally decreased as the saflufenacil became more bioavailable with time. In general, radioactive residues in tissues were rather low. Radioactivity generally declined continuously in organs and tissues of both sexes during the following hours in parallel to the concentration in plasma. After 20–24 hours, only negligible radioactivity was measured in internal organs except for the liver. By 168 hours, all internal tissues in male and female rats that received a single oral dose of ^{14}C-labelled saflufenacil at 5 or 100 mg/kg bw contained very low radioactivity (< 0.1 and < 1.0 µg equivalent [eq] per gram at 5 and 100 mg/kg bw, respectively; Tables 3, 4 and 5). Similar findings of low radioactivity in internal tissues (< 0.5 µg eq/g except for the liver of females) were also observed in male and female rats that were given 15 daily oral doses of saflufenacil at 100 mg/kg bw and sacrificed 168 hours later.

The amount of radioactivity excreted in the bile duct–cannulated rats following a single gavage dose of 5 or 100 mg/kg bw is shown in Table 6. Within 48 hours after administration of ^{14}C-labelled saflufenacil at the high dose level of 100 mg/kg bw, excretion via bile was found to be about 67.80% and 35.47% of the administered dose in male and female animals, respectively. Within 48 hours after administration of ^{14}C-labelled saflufenacil at a dose of 5 mg/kg bw, excretion via bile was found to be about 52.30% and 18.85% of the administered dose in male and female animals, respectively. There were sex-related differences in the excretion of orally administered saflufenacil, regardless of the dose. Generally, males excreted more radioactivity in the bile compared with females. Based on the amounts of radioactivity excreted via bile and urine, the bioavailability of ^{14}C-labelled saflufenacil in rats was virtually 100% at a dose of 100 mg/kg bw. At 5 mg/kg bw, the bioavailability of ^{14}C-labelled saflufenacil was virtually 100% in female rats and about 79% in male rats. The sum of urinary and biliary excretion is over 100% irrespective of dose in females and over 100% for the high dose level in males, suggesting significant enterohepatic circulation of saflufenacil (Fabian & Landsiedel, 2007a).

Table 2. Mean excretion and tissue retention of radioactivity after oral administration of [phenyl-^{14}C]saflufenacil

Sampling time (h)	% of administered dose					
	Males (n = 4 per dose group)			Females (n = 4 per dose group)		
	Dose (mg/kg bw)					
	5	100	100 (14 + 1)	5	100	100 (14 + 1)
Mean urinary radioactivity						
0–6	6.11 ± 3.31	28.10 ± 12.44	39.78 ± 4.26	30.96 ± 20.07	57.08 ± 6.49	55.99 ± 4.77
6–12	3.61 ± 2.94	9.53 ± 2.89	10.03 ± 4.73	15.38 ± 7.77	8.72 ± 1.90	6.23 ± 1.01
12–24	6.50 ± 5.46	10.26 ± 3.97	8.73 ± 1.09	29.55 ± 11.02	14.11 ± 4.21	10.78 ± 1.33
24–48	5.37 ± 3.97	3.26 ± 1.44	1.91 ± 0.52	14.06 ± 6.90	3.38 ± 1.59	5.25 ± 1.65
48–72	2.40 ± 2.55	0.79 ± 0.25	0.63 ± 0.22	3.56 ± 2.23	1.25 ± 0.64	2.09 ± 0.79
72–96	1.20 ± 1.61	0.34 ± 0.17	0.22 ± 0.05	1.11 ± 0.42	0.71 ± 0.43	1.11 ± 0.40
96–120	0.36 ± 0.28	0.16 ± 0.07	0.13 ± 0.04	0.56 ± 0.31	0.54 ± 0.32	0.63 ± 0.06
120–144	0.17 ± 0.13	0.10 ± 0.05	0.08 ± 0.03	0.45 ± 0.39	0.42 ± 0.09	0.67 ± 0.16
144–168	0.11 ± 0.07	0.06 ± 0.03	0.29 ± 0.45	0.43 ± 0.28	0.36 ± 0.10	0.63 ± 0.33
Urine total	26.02 ± 16.06	52.61 ± 10.11	61.80 ± 3.63	96.06 ± 7.88	86.56 ± 2.68	83.38 ± 2.43
Mean faecal radioactivity						
0–24	30.6 ± 21.98	21.14 ± 6.66	24.31 ± 4.89	6.55 ± 1.46	7.93 ± 2.48	9.64 ± 3.06
24–48	30.05 ± 9.91	18.24 ± 7.43	9.84 ± 2.83	4.71 ± 3.37	1.34 ± 0.47	2.80 ± 0.98
48–72	6.95 ± 3.17	2.92 ± 0.71	1.02 ± 0.19	1.11 ± 0.68	0.24 ± 0.18	0.52 ± 0.24
72–96	11.21 ± 20.04	0.58 ± 0.34	0.30 ± 0.14	0.21 ± 0.15	0.10 ± 0.05	0.14 ± 0.08
96–120	0.72 ± 0.96	0.20 ± 0.12	0.12 ± 0.06	0.09 ± 0.05	0.07 ± 0.03	0.09 ± 0.03
120–144	1.61 ± 2.93	0.18 ± 0.08	0.07 ± 0.03	0.09 ± 0.07	0.05 ± 0.01	0.10 ± 0.06
144–168	0.07 ± 0.02	0.09 ± 0.04	0.10 ± 0.07	0.04 ± 0.03	0.09 ± 0.09	0.09 ± 0.03
Faecal total	81.20 ± 16.41	43.33 ± 8.11	35.76 ± 3.80	12.80 ± 4.38	9.82 ± 2.22	13.38 ± 2.44
Cage wash, 168	0.56 ± 0.59	0.69 ± 0.31	0.58 ± 0.28	1.34 ± 0.65	0.80 ± 0.25	1.69 ± 0.45
Carcass, 168	0.06 ± 0.01	0.07 ± 0.03	0.05 ± 0.01	0.10 ± 0.05	0.08 ± 0.04	0.05 ± 0.02
Skin, 168	0.02 ± 0.0	0.03 ± 0.02	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01
Liver, 168	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.06 ± 0.02	0.03 ± 0.02	0.05 ± 0.02
Gut content, 168	0.02 ± 0.0	0.03 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Kidneys, 168	0.01 ± 0.01	0.0	0.0	0.01 ± 0	0.0	0.0
Total recovery, 168	107.90 ± 3.59	96.77 ± 3.31	98.24 ± 0.15	110.41 ± 3.18	97.32 ± 1.18	98.60 ± 0.09

From Fabian & Landsiedel (2007a). Data taken from tables 1, 8–13, pp. 37, 43–48 of the study report.

Table 3. Distribution of radioactivity in rat tissues/organs after administration of ^{14}C-labelled saflufenacil

Tissue/organ	Mean tissue concentration (µg eq/g)															
	Single low dose (5 mg/kg bw)								Single high dose (100 mg/kg bw)							
	Males				Females				Males				Females			
	Time after dosing (h)															
	1	24	48	72	1	4	20	24	1	7	20	34	1	7	20	34
Blood cells	8.34	1.90	0.36	0.16	5.42	2.37	0.92	0.43	74.02	27.96	8.30	5.80	43.24	12.90	7.70	6.57
Plasma	30.43	9.26	1.71	0.66	18.73	8.07	2.92	1.74	222.22	108.00	49.96	29.86	181.82	69.32	24.40	23.55
Lung	8.16	2.77	0.64	0.19	5.07	2.97	0.97	0.67	205.54	30.51	13.53	8.33	60.07	22.56	9.21	8.24
Heart	5.04	1.50	0.34	0.12	4.45	1.55	0.56	0.35	79.40	31.97	9.39	6.01	40.16	13.61	4.14	4.52
Spleen	1.82	0.55	0.15	0.05	1.26	0.64	0.22	0.14	36.35	10.26	3.62	2.64	18.40	5.28	2.22	2.03
Kidney	8.00	2.05	0.45	0.18	8.71	4.39	0.93	0.72	197.89	79.29	13.75	8.62	142.66	31.41	9.09	7.34
Adrenal glands	5.98	1.51	0.42	0.13	3.30	1.83	0.62	0.49	80.99	23.06	9.20	5.00	46.63	15.72	5.45	4.98
Testes/ovaries	1.27	0.52	0.28	0.04	3.35	1.75	0.63	0.36	26.10	19.56	7.51	4.93	41.85	14.95	5.85	4.61
Uterus	—	—	—	—	1.14	2.02	0.74	0.51	—	—	—	—	54.64	22.35	8.10	7.04
Muscle	1.25	0.39	0.11	0.03	0.86	0.45	0.14	0.09	35.25	9.98	3.49	1.95	14.93	4.24	1.60	1.48
Brain	0.71	0.19	0.04	0.01	0.37	0.17	0.07	0.04	4.68	1.95	1.03	0.61	3.83	1.52	0.58	0.51
Adipose tissue	0.95	0.35	0.10	0.03	0.88	0.42	0.16	0.16	12.54	10.41	3.76	1.67	9.32	3.97	2.75	1.45
Bone	1.01	0.29	0.06	0.03	0.69	0.33	0.14	0.08	18.87	7.44	3.06	1.47	10.15	3.35	1.67	1.14
Bone marrow	3.21	0.99	0.17	0.11	1.93	1.02	0.40	0.24	49.80	21.28	9.27	3.87	37.66	14.25	7.13	4.34
Thyroid	9.72	4.23	0.52	0.29	6.02	2.05	1.00	0.59	79.28	46.16	11.59	8.60	85.62	29.22	11.51	9.54
Pancreas	2.22	0.76	0.24	0.08	1.66	0.85	0.31	0.23	42.07	13.61	5.44	3.00	25.78	8.40	3.56	2.76
Stomach content	66.23	0.13	0.07	0.05	70.23	26.31	0.31	0.25	1213.7	576.83	22.24	2.32	3513.5	422.18	2.54	8.79
Stomach	19.77	0.91	0.22	0.08	18.43	13.55	0.54	0.38	421.61	137.66	12.43	3.69	483.67	157.55	4.30	4.55
Gut content	8.01	21.35	4.03	1.65	6.52	9.47	5.82	3.39	318.65	503.76	277.93	159.87	417.29	299.40	78.83	44.27
Gut	7.94	6.45	1.76	0.65	5.70	3.40	1.07	1.10	130.46	62.00	28.78	8.69	153.13	40.04	14.72	5.14
Liver	34.02	11.45	3.08	0.77	38.32	27.09	6.22	5.76	223.48	116.55	44.87	38.08	187.75	86.74	38.90	35.61
Skin	2.68	1.14	0.29	0.10	1.86	1.23	0.49	0.45	54.99	23.58	9.68	5.73	35.84	13.36	6.70	4.56
Carcass	1.77	0.75	0.20	0.06	1.34	0.72	0.28	0.18	30.66	13.55	5.56	2.86	13.20	4.72	2.11	1.69

From Fabian & Landsiedel (2007a). Data extracted from pp. 39–40 of the study report.

Table 4. Mean radioactivity in tissues after oral administration of ^{14}C-labelled saflufenacil at 5 mg/kg bw

	Mean radioactivity ± standard deviation (µg eq/g tissue)							
	Males (n = 3 per sacrifice interval)				Females (n = 3 per sacrifice interval)			
	Time (h)							
	1	24	48	72	1	4	20	24
Stomach content	66.2 ± 11.8	—	—	—	70.2 ± 22.6	26.3 ± 12.7	—	—
Liver	34.0 ± 3.01	11.5 ± 0.69	3.08 ± 3.45	—	38.3 ± 9.62	27.1 ± 5.98	6.22 ± 1.44	5.76 ± 2.14
Plasma	30.4 ± 3.42	9.26 ± 7.39	1.71 ± 1.97	—	18.7 ± 11.4	8.07 ± 4.76	2.92 ± 0.01	1.74 ± 2.09
Stomach	19.8 ± 1.53	—	—	—	18.4 ± 4.91	13.6 ± 6.90	—	—
Thyroid	9.72 ± 1.90	4.23 ± 3.20	—	—	6.02 ± 3.21	2.05 ± 1.73	1.00 ± 0.09	—
Blood cells	8.34 ± 2.07	1.90 ± 1.19	—	—	5.42 ± 2.49	2.37 ± 1.01	—	—
Lungs	8.16 ± 1.25	2.77 ± 1.92	—	—	5.07 ± 2.11	2.97 ± 1.41	—	—
Gut content	8.01 ± 0.29	21.4 ± 3.36	4.03 ± 2.01	1.65 ± 1.13	6.52 ± 0.50	9.47 ± 1.30	2.82 ± 1.16	3.39 ± 1.49
Kidneys	8.00 ± 0.58	2.05 ± 0.78	—	—	8.71 ± 1.32	4.39 ± 0.12	—	—
Gut	7.94 ± 0.69	6.45 ± 0.88	1.76 ± 1.06	—	5.70 ± 0.37	3.40 ± 0.52	1.07 ± 0.43	1.10 ± 0.65
Adrenals	5.98 ± 1.44	1.51 ± 1.01	—	—	3.30 ± 1.62	1.83 ± 1.04	—	—
Heart	5.04 ± 0.52	1.50 ± 1.09	—	—	4.45 ± 3.48	1.55 ± 0.72	—	—
Bone marrow	3.21 ± 0.44	—	—	—	1.93 ± 0.89	1.02 ± 0.63	—	—
Skin	2.68 ± 0.35	1.14 ± 0.88	—	—	1.86 ± 0.42	1.23 ± 0.68	—	—
Pancreas	2.22 ± 0.24	—	—	—	1.66 ± 0.69	—	—	—
Spleen	1.82 ± 0.16	—	—	—	1.26 ± 0.44	—	—	—
Carcass	1.77 ± 0.14	—	—	—	1.34 ± 0.47	—	—	—
Testes/ovaries	1.27 ± 0.22	—	—	—	3.35 ± 1.74	1.75 ± 0.72	—	—
Uterus	—	—	—	—	1.14 ± 1.30	2.02 ± 0.66	—	—
Muscle	1.25 ± 0.13	—	—	—	—	—	—	—
Bone	1.01 ± 0.11	—	—	—	—	—	—	—

From Fabian & Landsiedel (2007a). Data taken from tables 5, 30–33, pp. 40, 65–68 of the study report.

[a] Only tissues with ≥ 1.00 µg eq/g are listed.

Table 5. Mean radioactivity in tissues after oral administration of ^{14}C-labelled saflufenacil at 100 mg/kg bw[a]

	Mean radioactivity ± standard deviation (µg eq/g tissue)							
	Males (n = 3 per sacrifice interval)				Females (n = 3 per sacrifice interval)			
	Time (h)							
	1	7	20	34	1	7	20	34
Stomach content	1214 ± 471	577 ± 96.4	22.2 ± 27.7	—	3514 ± 1279	422 ± 258	—	8.79 ± 10.8
Liver	224 ± 45.3	117 ± 35.6	44.9 ± 7.31	38.1 ± 3.9	188 ± 7.87	86.7 ± 7.39	38.9 ± 9.17	35.6 ± 5.35
Plasma	222 ± 40.7	108 ± 43.9	50 ± 35	29.9 ± 7.01	182 ± 14.5	69.3 ± 9.62	24.4 ± 17.4	23.6 ± 9.72
Stomach	421.6 ± 121	138 ± 42.4	12.4 ± 10.4	—	484 ± 184	158 ± 188	—	—
Blood cells	74.0 ± 23.0	28.0 ± 10.2	8.30 ± 5.59	5.80 ± 2.15	43.2 ± 2.66	12.9 ± 5.7	7.70 ± 5.94	6.57 ± 2.44
Thyroid	79.3 ± 21.6	46.2 ± 17.3	11.6 ± 7.33	8.60 ± 1.53	85.6 ± 20.8	29.2 ± 3.70	11.5 ± 8.55	9.54 ± 3.00
Lungs	206 ± 237	30.5 ± 11.8	13.5 ± 9.29	8.33 ± 1.84	60.1 ± 11.2	22.6 ± 5.01	9.21 ± 6.15	8.24 ± 2.51
Kidneys	198 ± 19.2	79.3 ± 36.7	13.8 ± 5.83	8.62 ± 0.67	143 ± 8.90	31.4 ± 3.48	9.09 ± 3.10	7.34 ± 0.48
Gut	131 ± 27.8	62.0 ± 15.1	28.8 ± 15.8	8.69 ± 3.28	153 ± 29.4	40.0 ± 3.35	14.7 ± 3.63	5.14 ± 1.14
Gut content	319 ± 13.9	504 ± 83.0	278 ± 160	160 ± 82.3	417 ± 94.7	299 ± 68.6	78.8 ± 12.4	44.3 ± 13.9
Adrenals	81.0 ± 55.0	23.1 ± 8.81	9.20 ± 5.46	5.00 ± 0.43	46.6 ± 8.43	15.7 ± 2.11	5.45 ± 2.88	—
Heart	79.4 ± 30.2	32.0 ± 20.0	9.39 ± 6.22	6.01 ± 1.78	40.2 ± 6.04	13.6 ± 2.00	—	—
Bone marrow	49.8 ± 24.5	21.3 ± 7.21	9.27 ± 5.82	—	37.7 ± 6.76	14.2 ± 3.61	7.13 ± 5.64	—
Pancreas	42.1 ± 16.2	13.6 ± 5.55	5.44 ± 3.33	—	25.8 ± 6.40	8.40 ± 0.82	—	—
Spleen	36.4 ± 15.2	10.3 ± 3.33	—	—	18.4 ± 2.13	5.28 ± 0.54	—	—
Testes/ovaries	26.1 ± 7.78	19.6 ± 3.76	7.51 ± 4.86	—	41.9 ± 9.00	15.0 ± 3.25	5.85 ± 3.67	—
Uterus	—	—	—	—	54.6 ± 11.4	22.4 ± 5.42	8.10 ± 5.04	7.04 ± 2.01
Skin	55.0 ± 19.9	23.6 ± 9.75	9.68 ± 4.68	5.73 ± 0.92	35.8 ± 6.06	13.4 ± 1.83	6.70 ± 1.65	—
Carcass	30.7 ± 12.5	13.6 ± 3.28	5.56 ± 3.29	—	13.2 ± 0.85	—	—	—

From Fabian & Landsiedel (2007a). Data taken from tables 4, 21–24, pp. 39, 56–59 of the study report.

[a] Only tissues with ≥ 5.00 µg eq/g are listed.

Table 6. Percentages of biliary excretion of radioactivity after a single oral administration of ^{14}C-labelled saflufenacil at 5 or 100 mg/kg bw

Time interval (h)	% of biliary excretion (mean ± standard deviation)			
	Males		Females	
	Dose (mg/kg bw)			
	5	100	5	100
0–3	2.82 ± 1.91	5.01 ± 3.41	1.10 ± 0.34	2.14 ± 1.16
3–6	4.59 ± 2.66	8.15 ± 6.47	1.09 ± 0.21	1.78 ± 0.72
6–9	4.69 ± 2.29	6.59 ± 5.21	0.81 ± 0.21	1.46 ± 0.64
9–12	5.33 ± 2.24	6.24 ± 3.58	0.77 ± 0.14	1.94 ± 1.18
12–15	5.99 ± 2.01	6.94 ± 1.02	0.77 ± 0.07	3.41 ± 2.44
15–18	5.99 ± 1.16	7.60 ± 1.68	0.91 ± 0.15	3.20 ± 1.05
18–21	4.64 ± 0.93	5.29 ± 1.47	1.24 ± 0.21	3.29 ± 0.88
21–24	3.32 ± 0.81	4.28 ± 2.69	1.23 ± 0.47	3.18 ± 0.75
24–27	2.52 ± 0.61	3.36 ± 2.13	0.98 ± 0.71	3.11 ± 0.90
27–30	2.21 ± 0.53	2.64 ± 1.63	0.88 ± 0.88	2.70 ± 1.21
30–33	2.01 ± 0.39	2.26 ± 1.36	1.03 ± 0.55	2.35 ± 1.14
33–36	1.76 ± 0.27	2.41 ± 2.08	1.58 ± 0.45	2.11 ± 0.84
36–39	1.62 ± 0.55	2.44 ± 2.48	1.63 ± 0.42	1.69 ± 0.59
39–42	1.63 ± 0.38	1.88 ± 1.82	1.76 ± 0.36	1.33 ± 0.42
42–45	1.71 ± 0.48	1.47 ± 1.29	1.51 ± 0.24	0.87 ± 0.23
45–48	1.47 ± 0.55	1.18 ± 1.01	1.13 ± 0.43	0.92 ± 0.15
Total	52.3 ± 13.4	67.8 ± 6.16	18.4 ± 3.36	35.5 ± 4.94

From Fabian & Landsiedel (2007a). Data taken from tables 40–41, pp. 75–76 of the study report.

1.2 Biotransformation

Urine, faeces and bile samples collected from the previously conducted absorption, distribution and excretion studies in rats following a single oral dose of 5 or 100 mg/kg bw and a 15-day repeated-dosing study at 100 mg/kg bw were used for metabolic characterization (Fabian & Landsiedel, 2007a). Additional metabolism studies were conducted for the isolation and identification of urinary and faecal metabolites. In these studies, Wistar rats (10 of each sex per dose) were administered a single gavage dose of [uracil-^{14}C]saflufenacil or [phenyl-^{14}C]saflufenacil at 100 mg/kg bw. Urine and faecal samples were collected at several time points for up to 168 hours. Animals were sacrificed 168 hours following dosing. For the analysis of the metabolite patterns in liver, kidney, fat and plasma, Wistar rats (four of each sex per dose) were administered a single gavage dose at 5 or 100 mg/kg bw (uracil and phenyl label), and the organs were removed 1 hour after dosing. Appropriate aliquots of the pooled urine and bile samples were directly subjected to radio-high-performance liquid chromatography (HPLC) without further extraction or other kind of workup. Faecal samples were extracted 3 times with acetonitrile and concentrated with a rotary evaporator. Aliquots of acetonitrile extracts were measured by liquid scintillation counting (LSC) and HPLC. Liver and kidney samples were homogenized and extracted 3 times with acetonitrile and analysed by LSC and HPLC. Aliquots of homogenized fat samples were extracted either 2 or 3 times with a mixture of acetonitrile and isohexane (50:50 volume per volume [v/v]). After centrifugation, the supernatants were transferred to a separatory funnel. The separated phases of acetonitrile and isohexane were collected in volumetric flasks and each adjusted to a defined volume. The acetonitrile extracts were combined, concentrated

with a rotary evaporator and adjusted to a defined volume, and aliquots were measured by LSC and HPLC. Residues were treated with Biolute S and water. The solution was kept at 50 °C until the tissue was completely dissolved. Afterwards, Lumagel Plus was added, and the sample was stored overnight in the refrigerator until chemoluminescence decayed. Radioactivity was determined by LSC. The metabolites were identified using various analytical methods, such as HPLC, mass spectrometry, nuclear magnetic resonance (NMR) spectrometry and co-chromatography (Grosshans, 2007).

The unchanged parent compound was predominant in the urine of all dose groups. In the urine, saflufenacil amounted to 10.9–48.2% of the administered dose for male rats and to 43.9–88.9% of the administered dose for female rats (Tables 7 and 8). The major metabolites identified in urine samples of male rats (Tables 7 and 8) were M800H01 (3.5–9.1% of the administered dose), M800H03 (0.8–2.3% of the administered dose), M800H05 (0.4–4.2% of the administered dose) and M800H07 (0.4–2.2% of the administered dose). In urine samples of female rats (Tables 7 and 8), the metabolite M800H07 (0.6–4.6% of the administered dose) was the predominant one; the other metabolites amounted to 0.05–1.7% of the administered dose. M800H09 occurred in small amounts (< 0.01–0.3% of the administered dose) in urine of both males and females. M800H37 (a phenyl label–specific metabolite), M800H23 (a uracil label–specific metabolite), M800H02 (both phenyl and uracil label) and M800H06 (both phenyl and uracil label) were also detected in small amounts in the urine of male and female rats. Total identified metabolites in urine (both labels) were in the range of 25–62% of the dose for male rats and 53.4–95% of the dose for female rats (Tables 7 and 8).

The predominant metabolites in faeces of male and female rats in all dose groups were M800H01 (18.1–43.9% of the administered dose in males and 1.0–2.5% of the administered dose in females) and the unchanged parent compound (3.8–16.2% and 2.9–9.9% of the administered dose in males and females, respectively) (Tables 7 and 8). Other metabolites identified in faeces were M800H02, M800H03 (male = 2.1–5.2% of the administered dose, female = 0.4–1.6% of the administered dose), M800H04, M800H05 (male = 0.3–3.6% of the administered dose, female = < 0.01–0.2% of the administered dose), M800H06, M800H07 (male = 1.1–2.0% of the administered dose, female = 0.7–1.4% of the administered dose) and M800H08. The HPLC retention behaviour of the metabolites M800H02 and M800H06, as well as of the metabolites M800H04 and M800H08, were very similar; therefore, the peaks were not clearly separated by HPLC. The sum of M800H02 and M800H06 amounted to 2.4–8.6% of the administered dose for male rats and 0.2–0.6% of the administered dose for female rats. The sum of M800H04 and M800H08 amounted to 0.7–2.4% of the administered dose for male rats and to 0.6–1.2% of the administered dose for female rats. The phenyl label–specific metabolite M800H37 was found in amounts of 0.8–4.5% and < 0.01–0.2% of the administered dose for male and female rats, respectively. Metabolite M800H09 was detected only in minor amounts (0.03% of the administered dose) in faeces of female rats. The uracil label–specific metabolite M800H23 was not detected in faeces of both sexes. Total identified metabolites in faeces were in the range of 32–72% of the administered dose for male rats and 10.3–13.4% of the administered dose for female rats (Tables 7 and 8).

For male rats, the major metabolites in the bile (Table 9) were M800H01 (8.7–10.6% of the administered dose), M800H07 (11.1–13.4% of the administered dose), M800H18 (8.9–11.5% of the administered dose) and the unchanged parent compound (4.8–14.5% of the administered dose). The metabolite M800H18 was identified as a derivative of the parent compound in which the uracil ring was cleaved to form the *N*-sulfonylamide group, combined with a demethylation of the *N*-methyl-*N*-isopropyl group. Other metabolites identified in the bile were M800H02 + M800H17 (2.1–4.4% of the administered dose), M800H16 (1.6–2.4% of the administered dose), M800H19 (3.1–3.2% of the administered dose), M800H20 (4.1–4.6% of the administered dose) and M800H21 (0.2–0.3% of the administered dose). M800H17 was identified as a derivative of the parent compound in which the uracil ring was cleaved, but the three-carbon fragment remained attached to the molecule. M800H20 and M800H19 were formed by further degradation of the *N*-methyl-*N*-isopropyl group. Metabolite M800H16

Table 7. Identified metabolites in urine and faeces of rats 72–168 hours after single oral administration of ^{14}C-labelled saflufenacil (phenyl label) at 5 mg/kg bw and 48–168 hours after repeated daily administration of ^{14}C-labelled saflufenacil (phenyl label) at 100 mg/kg bw

Metabolite	% of administered dose							
	Single 5 mg/kg bw				14 + 1 doses; 100 mg/kg bw per day			
	Urine		Faeces		Urine		Faeces	
	Males	Females	Males	Females	Males	Females	Males	Females
	Time assessed (h)							
	0–168	0–168	0–96	0–72	0–168	0–168	0–72	0–48
Saflufenacil	10.9	88.9	3.77	2.93	48.2	78.1	5.55	5.25
M800H01	5.21	0.85	43.9	2.52	7.88	1.70	18.08	1.63
M800H05	4.23	0.09	3.55	0.14	1.45	—	0.867	0.09
M800H03	2.34	0.67	5.22	1.63	1.96	0.43	2.092	0.84
M800H07	1.65	4.62	2.01	1.39	1.86	2.54	1.136	1.12
M800H37	0.54	0.01	4.50	0.13	0.16	—	1.015	0.20
M800H02+06	0.04	—	8.64	0.58	0.04	—	2.387	0.51
M800H09	0.02	—	—	0.03	0.002	0.02	—	—
M800H04+08	nd	—	0.68	0.97	nd	—	1.177	1.23
Total identified	25.0	95.2	72.3	10.3	61.6	82.7	32.3	10.9
Total unidentified	1.05	0.91	3.79	1.08	0.19	0.64	1.31	1.27

From Grosshans (2007). Data taken from tables 10–13, 18–21, pp. 63–65, 67–69 of the study report.
nd, not detectable; —, not reported

could be formed by oxidation of M800H17. M800H21 was an oxidation product of BAS 800 H. In female rats, the predominant biliary metabolite (Table 9) was M800H07 (4.8–8.1% of the administered dose) and the parent compound (6.6–11.4% of the administered dose). Other identified minor metabolites were M800H01 (0.7–1.1% of the administered dose), M800H02 + M800H17 (1.8–3.1% of the administered dose), M800H16 (0.8–1.7% of the administered dose), M800H18 (0.6–1.1% of the administered dose), M800H19 (0.8–2.1% of the administered dose), M800H20 (0.7–1.4% of the administered dose) and M800H21 (0.1–0.3% of the administered dose). Total identified metabolites in bile were in the range of 47–62% and 17–31% of the administered dose in male and female rats, respectively.

In liver, saflufenacil was identified in the range of 16–25% of the dose for the low-dose group and in the range of 4–7% of the dose for the high-dose group (both labels, not sex specific). The metabolite M800H04 amounted to 1.6–4.3% of the dose for the low-dose group and to 1.3–2.1% of the dose for the high-dose group (both labels, not sex specific). The metabolite M800H0l was detected in amounts less than or equal to 1.3% of the dose. The metabolite patterns in kidney and fat were comparable to those in liver. The total radioactivity and the sum of all identified metabolites were below or equal to 1.0% of the dose. In plasma, saflufenacil (1.6–4.1% of the dose) appeared to be predominant throughout all dose groups. M800H01, M800H04 and M800H07 were identified in minor amounts (each below 0.2% of the dose).

In rats, saflufenacil was metabolized by three major transformation steps, which are demethylation of the uracil ring system, degradation of the *N*-methyl-*N*-isopropyl group to amine and cleavage of the uracil ring, forming a sulfonylamide group. The predominant compounds were the metabolites M800H01, M800H03 and M800H07 and the parent compound for male and female rats. In addition, the metabolites M800H05 and M800H18 also occurred in male rats. The metabolites M800H01,

Table 8. Identified metabolites in urine and faeces of rats 24–168 hours after oral administration of ^{14}C-labelled saflufenacil at 100 mg/kg bw

Metabolite	% of administered dose																	
	Urine									Faeces								
	Males					Females				Males					Females			
	Time interval (h)																	
	0–24		0–96		0–168	0–24	0–96	0–168		0–24		0–72	0–96		0–24	0–48	0–72	
	P	U	P	U	P	P	P	P	U	P	U	P	P	U	P	P	P	U
Saflufenacil	21.7	20.9	23.1	22.2	36.6	43.9	48.7	82.1	53.3	14.6	13.7	4.84	16.0	16.2	7.54	3.75	9.94	9.20
M800H01	2.95	3.39	3.53	3.93	9.05	nd	0.06	0.47	0.05	13.2	12.9	23.0	22.3	25.8	0.52	1.16	0.98	1.27
M800H05	0.50	0.29	0.69	0.44	1.76	—	—	nd	—	0.29	nd	0.77	0.59	0.33	—	0.18	—	—
M800H03	0.59	0.63	0.75	0.81	2.21	nd	0.04	0.05	—	1.66	1.52	2.40	2.65	2.71	0.15	0.65	0.36	0.45
M800H07	0.28	—	0.40	—	2.16	0.42	0.63	3.64	—	0.85	—	1.41	1.20	—	0.30	0.75	0.76	—
M800H37	—	—	0.02	—	0.19	—	—	0.01	—	0.47	—	1.79	0.83	—	—	0.10	—	—
M800H02+06	nd	nd	nd	nd	—	nd	nd	—	—	1.32	1.13	2.90	2.68	3.20	nd	0.32	0.16	0.36
M800H09	—	0.30	—	0.34	—	—	—	0.04	0.01	—	nd	—	—	nd	—	—	—	—
M800H04+08	nd	nd	nd	nd	—	nd	nd	—	—	0.97	1.52	0.95	1.38	2.37	0.61	0.59	1.24	0.78
M800H23	—	0.26	—	0.38	—	—	—	—	0.05	—	nd	—	—	nd	—	—	—	—
Total	26.0	25.8	28.5	28.1	52.0	44.3	49.5	86.3	53.4	33.3	30.8	38.1	47.6	50.6	9.12	7.50	13.4	12.1

From Grosshans (2007). Data taken from tables 14–17, 22–26, pp. 65–67, 70–72 of the study report.
nd, not detectable; —, not reported; P, phenyl label; U, uracil label

Table 9. Identified metabolites in the bile of bile duct–cannulated rats 48–72 hours after single administration of [phenyl-^{14}C]saflufenacil at 5 or 100 mg/kg bw

Metabolite	% of administered dose			
	5 mg/kg bw		100 mg/kg bw	
	Males	Females	Males	Females
	Time interval (h)			
	0–48	0–48	0–48	0–48
Saflufenacil	4.78	6.56	14.5	11.4
M800H18	11.5	0.61	8.87	1.14
M800H07	11.1	4.77	13.4	8.08
M800H01	8.69	0.72	10.6	1.13
M800H20	4.11	0.70	4.58	1.43
M800H02+17	2.08	1.75	4.42	3.08
M800H16	1.62	0.82	2.43	1.67
M800H21	0.17	0.14	0.26	0.32
M800H19	3.06	0.83	3.21	2.06
Total	47.0	16.9	62.2	30.9

From Grosshans (2007). Data taken from tables 27–30, pp. 73–76 of the study report.

M800H03 and M800H05 were identified as derivatives of saflufenacil in which the *N*-methyl-*N*-isopropyl group was step by step degraded to unsubstituted sulfonamide. The metabolite M800H07 was identified as a derivative of the parent compound in which the uracil ring was cleaved with loss of three carbons to form a phenyl-*N*-methylurea group. The metabolite M800H18 was identified as a derivative of the parent compound in which the uracil ring was cleaved to form a phenyl-*N*-methyl-urea, combined with demethylation of the *N*-methyl-*N*-isopropylsulfonamide. Metabolites M800H16, M800H17, M800H18, M800M19 and M800M20 were present in bile only at remarkable portions. Further identified metabolites were M800H02, M800H04, M800H06, M800H08, M800H09, M800H10, M800H11, M800H16, M800H17, M800H19, M800H20, M800H21, M800H23, M800H35 and M800H37. The summary of identified metabolites found in urine, faeces, bile, liver, kidney, fat and plasma is shown in Table 10. The proposed metabolic pathway of saflufenacil (BAS 800 H) in rat is shown in Figure 2 (Grosshans, 2007).

2. Toxicological studies

2.1 Acute toxicity

The acute toxicity of saflufenacil is summarized in Table 11.

(a) Oral administration

Rats

Three fasted female young adult Wistar rats were treated orally, by gavage, with saflufenacil (purity 93.8%) in 0.5% carboxymethylcellulose in double-distilled water at a single dose of 2000 mg/kg bw. As no morbidity or mortality was observed in the first test group, a second group of three fasted female young adult Wistar rats was similarly treated at a single gavage dose of 2000 mg/kg bw. Animals were observed for mortality and clinical signs several times for the 1st day and once daily thereafter for 13 days. Body weights were recorded on days 0, 7 and 14. A gross necropsy was performed on all animals. The stability of the test substance was confirmed analytically.

Table 10. Summary of identified metabolites in urine, faeces, bile, liver, kidney, fat and plasma of rats

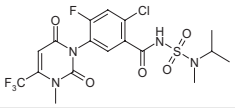
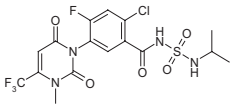
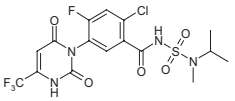
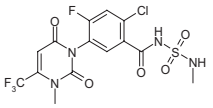
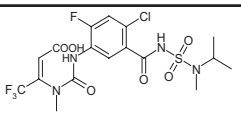
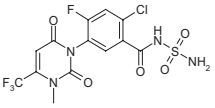
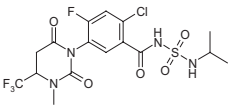
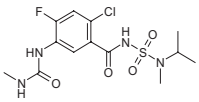
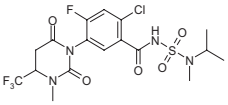
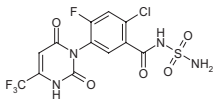
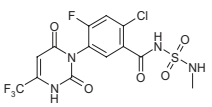
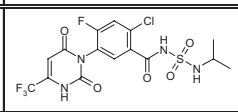
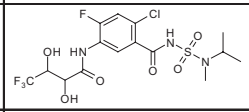
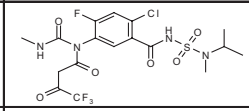
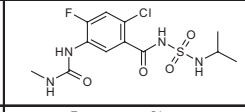
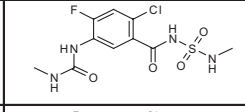
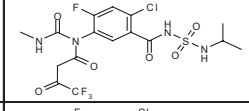
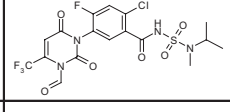
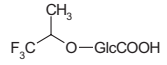
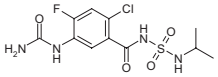
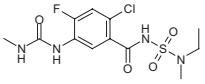
Metabolite Designation	Molecular Mass	Structure	Urine		Feces		Bile	Liver		Kidney		Fat		Plasma	
			phenyl-label	uracil-label	phenyl-label	uracil-label	phenyl-label	phenyl-label	uracil-label	phenyl-label	uracil-label	phenyl-label	uracil-label	phenyl-label	uracil-label
BAS 800 H	500		x	x	x	x	x	x	x	x	x	x	x	x	x
M800H01	486		x	x	x	x	x	x	x	x	x	x	x	x	-
M800H02	486		x	x	x	x	x	-	-	-	-	-	-	-	-
M800H03	458		x	x	x	x	-	x	-	x	-	-	-	-	-
M800H04	518		-	-	x	x	-	x	x	x	x	x	x	x	x
M800H05	444		x	x	x	x	-	-	-	-	-	-	-	-	-
M800H06	488		x	x	x	x	-	-	-	-	-	-	-	-	-
M800H07	380		x	-	x	x[1]	x	-	-	x	-	-	-	-	-
M800H08	502		-	-	x	x	-	-	-	-	-	-	-	-	-
M800H09	430		x	x	x	-	-	-	-	-	-	-	-	-	-
M800H10*	444		-	x	-	-	-	-	-	-	-	-	-	-	-

Table 10 (continued)

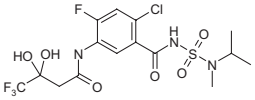
Metabolite Designation	Molecular Mass	Structure	Urine		Feces		Bile	Liver		Kidney		Fat		Plasma	
			phenyl-label	uracil-label	phenyl-label	uracil-label	phenyl-label	phenyl-label	uracil-label	phenyl-label	uracil-label	phenyl-label	uracil-label	phenyl-label	uracil-label
M800H11*	472		x	-	x	-	x	-	-	-	-	-	-	-	-
M800H16	479		-	-	-	-	x	-	-	-	-	-	-	-	-
M800H17	518		-	-	-	-	x	-	-	-	-	-	-	-	-
M800H18	366		-	-	-	-	x	-	-	-	-	-	-	-	-
M800H19	338		-	-	-	-	x	-	-	-	-	-	-	-	-
M800H20	504		-	-	-	-	x	-	-	-	-	-	-	-	-
M800H21	514		-	-	-	-	x	-	-	-	-	-	-	-	-
M800H23	290		-	x	-	-	-	-	-	-	-	-	-	-	-
M800H35*	352		-	-	-	-	x	-	-	-	-	-	-	-	-
M800H37	366		x	-	x	-	-	-	-	-	-	-	-	-	-

Notes: * Metabolites were identified by liquid chromatography–tandem mass spectrometry (LC-MS/MS). Quantification within the study was not feasible because of negligible amounts.

x = identified

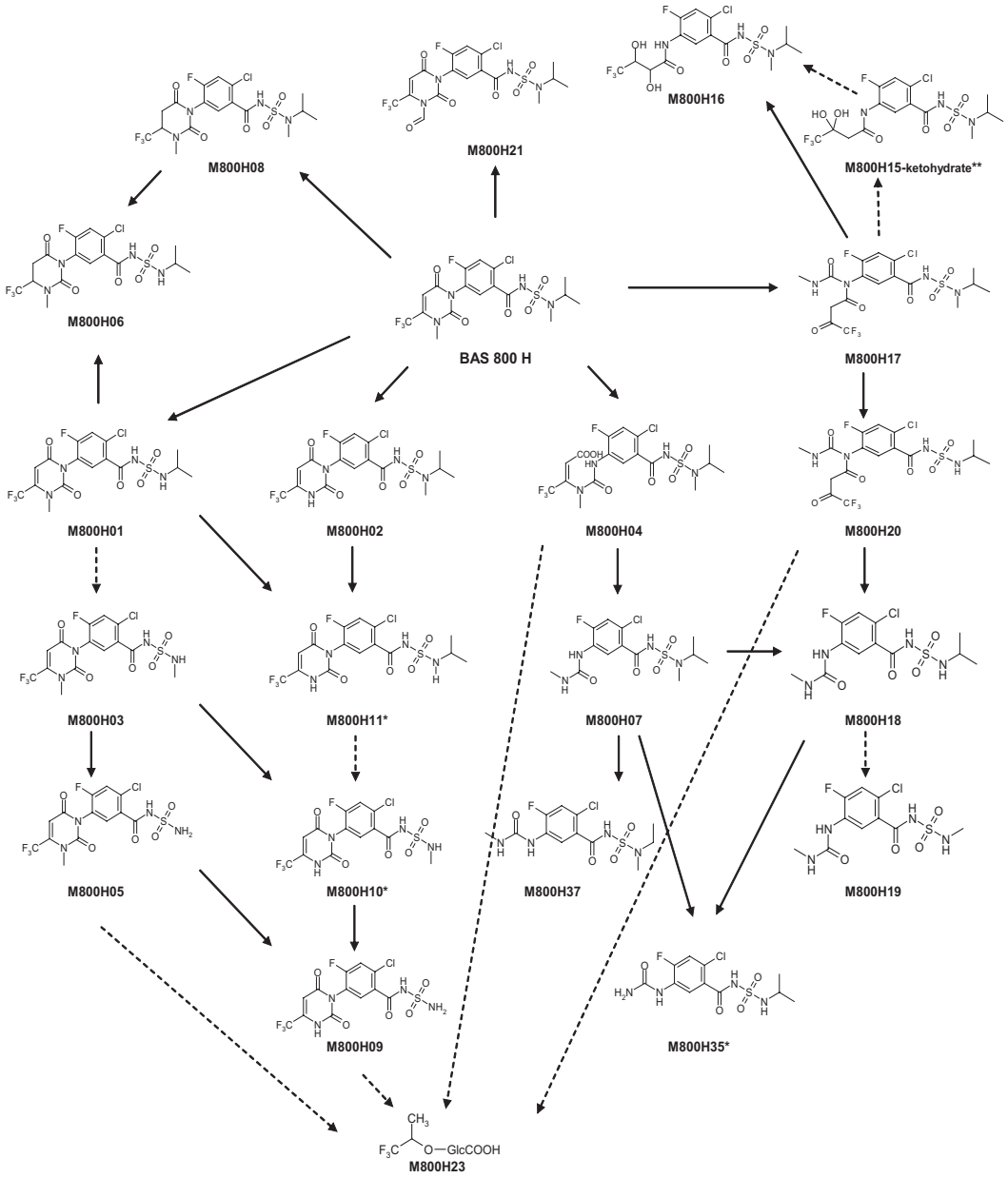
- = not identified

M800H15-ketohydrate is a potential intermediate not identified during the current study.

M800H15-ketohydrate: 

[1] Metabolite M800H07 was detected in the faecal extract from the uracil label by LC-MS/MS only and not by radio-HPLC.

Figure 2. Proposed metabolic pathway of saflufenacil (BAS 800 H) in the rat



* Metabolites were identified by LC-MS/MS. Radio-detection and thus quantification within the study were not feasible because of negligible amounts.

** Metabolite M800H15-ketohydrate is a potential intermediate not identified in the study.

Table 11. Acute toxicity of saflufenacil

Species	Strain	Sex	Route	Purity (%)	Results	Reference
Rat	Wistar	Female	Oral	93.8	LD_{50} > 2000 mg/kg bw	Gamer & Leibold (2005a)
Rat	Wistar	Male and female	Dermal	93.8	LD_{50} > 2000 mg/kg bw	Gamer & Leibold (2005b)
Rat	Wistar	Male and female	Inhalation (nose only)	93.8	LC_{50} > 5.3 mg/l	Ma-Hock & Leibold (2005)
Rabbit	New Zealand White	Male and female	Dermal irritation	93.9	Non-irritating	Remmele & Leibold (2005b)
Rabbit	New Zealand White	Male and female	Ocular irritation	93.9	Minimally irritating	Remmele & Leibold (2005a)
	New Zealand White	Male	Ocular irritation	93.8	Minimally irritating	Remmele & Landsiedel (2007)
Guinea-pig	Dunkin Hartley	Female	Dermal sensitization (maximization test)	93.8	Non-sensitizing	Gamer & Leibold (2005c)

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

No deaths or clinical signs of an adverse reaction to treatment occurred, and there were no macroscopic findings at necropsy in any animal. The mean body weights of the first group were increased more during the 1st week of observation than during the 2nd week. The mean body weights of the second group more uniformly increased over 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 (Gamer & Leibold, 2005a).

(b) Dermal application

Rats

A group of five male and five female young adult Wistar rats were treated with saflufenacil (purity 93.8%) dispersed in aqueous solution of 0.5% carboxymethylcellulose once for 24 hours by topical, semi-occluded application to a clipped area of intact dorsal skin (40 cm^2; approximately 10% of body surface area) at a dose level of 2000 mg/kg bw. Animals were observed for mortality and clinical signs several times for the 1st day and once daily thereafter for 13 days. Body weights were recorded on days 0, 7 and 14. The animals were observed for 14 days post-treatment and then subjected to necropsy and postmortem examination.

No deaths or systemic clinical signs of an adverse reaction to treatment occurred. There were no local signs of an effect of treatment at the application site, and there were no macroscopic findings at necropsy in any animal. All animals gained weight during the study. Based on these results, the acute dermal LD_{50} was estimated to be greater than 2000 mg/kg bw (Gamer & Leibold, 2005b).

(c) Exposure by inhalation

Rats

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 saflufenacil (purity 93.8%) at an analytically determined mean concentration of 5.3 mg/l. The animals were observed for 14 days post-treatment, during which time clinical signs were recorded twice a day during weekdays and once on weekends. Body weights were recorded on day 0 and weekly thereafter. All animals were subjected to necropsy and postmortem examination.

Cascade impactor measurements resulted in particle size distributions with mass median aerodynamic diameters (MMADs) of 2.3 and 3.2 μm, which were within the respirable range. No deaths occurred during the exposure or observation periods. Clinical signs of toxicity comprised squatting posture, piloerection, visually accelerated respiration and smeared and contaminated fur in all animals. Findings were observed from hour 0 of exposure until and including study day 3. The mean body weights of the male and female animals increased throughout the post-exposure observation period. There were no macroscopic findings at necropsy in any animal. Based on these results, the acute median lethal concentration (4-hour median lethal concentration [LC_{50}]) was estimated to be greater than 5.3 mg/l air (Ma-Hock & Leibold, 2005).

(d) Dermal irritation

Rabbits

In a study of primary dermal irritation, one male and two female young adult New Zealand White rabbits were dermally exposed to 0.5 g of saflufenacil (purity 93.9%) applied to a 2.5 cm × 2.5 cm patch, covered with a semi-occlusive dressing. The test material was in contact with the skin for 4 hours. The adjacent skin area served as the control. After removal of the patch, the treated application site was washed off with Lutrol and Lutrol/water (1:1). Dermal irritation was scored at 1, 24, 48 and 72 hours after the removal of the patch. The animals were observed for 14 days post-treatment, during which time clinical signs were recorded twice a day during weekdays and once on weekends. Body weights were recorded prior to treatment and at termination.

Slight erythema was observed in all animals up to 1 hour after removal of the patch. The cutaneous reactions were reversible in all animals within 24 hours after removal of the patch. The average score (24–72 hours) for irritation was calculated to be 0.0 for erythema and for oedema. Based on the results of this study, saflufenacil was not irritating to the skin of rabbits (Remmele & Leibold, 2005b).

(e) Ocular irritation

Rabbits

In a primary eye irritation study, approximately 0.1 g of saflufenacil (about 32 mg; purity 93.9%) was instilled into the right conjunctival sac of one female and two male New Zealand White rabbits. About 1 hour after application, treated eyes were rinsed with 3–6 ml of warm tap water for 1–2 minutes. The ocular irritation was assessed approximately 1, 24, 48 and 72 hours after application.

Slight or moderate conjunctival redness (grade 1 or 2) was observed in all animals up to 24 hours after application. Slight conjunctival chemosis (grade 1) was noted in one animal, and slight discharge (grade 1) was noted in all animals 1 hour after application. In addition, injected scleral vessels in a circumscribed area were noted in the animals up to 24 hours after application. The ocular reactions were reversible in all animals within 48 hours after application. The average score (24–72 hours) for irritation was calculated to be 0.0 for corneal opacity, iritis and chemosis and 0.3 for conjunctival redness. Based on the results of this study, saflufenacil is minimally irritating to rabbit eyes (Remmele & Leibold, 2005a).

In a second primary eye irritation study, approximately 0.1 g of saflufenacil (about 32 mg; purity 93.8%) was instilled into the right conjunctival sac of three male New Zealand White rabbits. About 24 hours after application, treated eyes were rinsed with 3–6 ml of warm tap water for 1–2 minutes. The ocular irritation was assessed approximately 1, 24, 48 and 72 hours after application.

Slight or moderate conjunctival redness, slight conjunctival chemosis and slight or moderate discharge were observed in the animals within 24 hours after application. In addition, injected

scleral vessels in a circumscribed area were noted in all animals 1 hour after application only. The ocular reactions were reversible in two animals within 24 hours and in one animal within 48 hours after application. The average score (24–72 hours) for eye irritation was calculated to be 0.0 for corneal opacity, iritis and chemosis and 0.1 for conjunctival redness. Based on the results of this study, saflufenacil is minimally irritating to rabbit eyes (Remmele & Landsiedel, 2007).

(f) Dermal sensitization

Guinea-pigs

The skin sensitization potential of saflufenacil (purity 93.8%) was investigated in female Dunkin Hartley guinea-pigs (10 test animals and 5 negative controls) using the maximization test. A concurrent positive control group was not included. Concentrations of 5%, 50% and 25% saflufenacil in aqueous carboxymethylcellulose solution 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 intradermal induction caused moderate and confluent to intense erythema and swelling at the injection sites of the test substance preparation in all test group animals. After the epicutaneous induction, incrustation, partially open (caused by the intradermal induction), could be observed in addition to moderate and confluent erythema and swelling in all test group animals. Saflufenacil was not sensitizing to skin in the maximization test in guinea-pigs (Gamer & Leibold, 2005c).

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

In a 28-day toxicity study, saflufenacil (purity 94.2%) was administered daily in the diet to C57BL/6NCrl mice (five of each sex per dose) at 0, 50, 150, 450, 1350 or 4050 parts per million (ppm) (equal to 0, 12.8, 36.6, 112, 335 and 882 mg/kg bw per day in males and 0, 17.9, 63.4, 153, 446 and 1621 mg/kg bw per day in females). The diets were analysed for stability, homogeneity and concentration. The animals were examined for clinical signs and mortality twice a day on working days and once daily on Saturdays, Sundays and public holidays. Detailed clinical observations were performed on all animals prior to the administration period and thereafter at weekly intervals. Body weight was determined before the start of the administration period in order to randomize the animals. During the administration period, the body weight was determined on day 0 and thereafter at weekly intervals. Individual feed consumption was determined weekly over a period of 7 days and calculated as mean feed consumption in grams per animal per day. An ophthalmoscopic examination was not conducted. Urine parameters were not analysed in this study. At the end of the study, blood was collected for haematology and clinical chemistry measurements. All rats that died and those sacrificed at study termination were subject to gross pathological examination, and the selected tissues were collected for histological examination. The selected organs were weighed.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable (98.2–102.8% of the nominal concentrations). No clinical signs of toxicity were observed. One female animal of the 150 ppm dose group was found dead on day 28 of the study, which was considered not related to the treatment. There was no treatment-related effect on body weight or body weight gain in females. The body weight and body weight gains of males at 1350 and 4050 ppm were adversely affected. At termination, the body weight gains of males were 63% and 31% of the control values at 1350 and 4050 ppm, respectively. There were no statistically significant findings for feed consumption, although males at 4050 ppm

consistently consumed less feed during the study period. In males, significantly lower values for red blood cells, haemoglobin, haematocrit, mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) were observed in males at 1350 and 4050 ppm. In addition, haemoglobin and haematocrit values were significantly lower in the male mice at 150 and 450 ppm, and MCV and MCH were significantly decreased in the males at 450 ppm. Examination of red blood cell morphology in males revealed increased anisocytosis, microcytosis and polychromasia at 4050 ppm. Increased polychromasia was also seen in the erythrocytes of the males at 1350 ppm. In females, significantly reduced values for haemoglobin, haematocrit, MCV, MCH and mean corpuscular haemoglobin concentration (MCHC) were found at 4050 ppm. Increased anisocytosis and polychromasia were measured in erythrocytes of females at 4050 ppm. Serum enzyme examinations revealed dose-dependent, significant increases in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities in males given 150, 450, 1350 and 4050 ppm of the test compound. Significantly higher alkaline phosphatase activities were also seen in males at 4050 ppm. In the serum of the females receiving 1350 and 4050 ppm of the test substance, ALT activities were significantly increased. No treatment-related changes were seen in the other serum enzymes or in cyanide-insensitive palmitoyl coenzyme A oxidation in the liver. Significantly increased urea and total bilirubin concentrations were found in the serum of males receiving 150, 450, 1350 and 4050 ppm of the test substance, and decreased glucose levels were noted in male mice at 450, 1350 and 4050 ppm. However, the fall in glucose level in the 1350 ppm group appeared only as a tendency towards reduced values. In males, there were statistically significant increases in absolute and relative liver weights at and above 150 ppm and an increase in spleen weight at 4050 ppm. All other statistically significant weight changes (kidneys, thymus and brain) were either secondary to the significant terminal body weight decrease in the high-dose group or of no biological significance. In females, the only treatment-related findings on organ weights were statistically significant increases of absolute and relative liver weights at 1350 and 4050 ppm. There were no treatment-related gross pathological findings. Substance-induced findings were observed in the liver and spleen. In the liver, changes included centrilobular fatty changes (males $\geq$ 150 ppm; females $\geq$ 450 ppm), minimal lymphoid infiltration (males $\geq$ 150 ppm; females $\geq$ 1350 ppm) and extramedullary haematopoiesis (males $\geq$ 450 ppm). In the spleen, the only finding was extramedullary haematopoiesis (males $\geq$ 1350 ppm; females 4050 ppm). A slight increase of apoptotic necrosis of lymphocytes was observed in the thymus of treated males at and above 150 ppm, which was regarded as a secondary effect of treatment.

The lowest-observed-adverse-effect level (LOAEL) established in males was 150 ppm (equal to 36.6 mg/kg bw per day), based on haematological parameters (decreased haemoglobin and haematocrit), altered clinical chemistry (increased ALT, AST, urea and total bilirubin) and liver pathology (increased weight and centrilobular fatty change). The no-observed-adverse-effect level (NOAEL) in males was 50 ppm (equal to 12.8 mg/kg bw per day). The LOAEL established in females was 450 ppm (equal to 153 mg/kg bw per day), based on moderate centrilobular fatty change in the liver. The NOAEL in females was 150 ppm (equal to 63.4 mg/kg bw per day) (Kaspers et al., 2007a).

In a 90-day toxicity study, saflufenacil (purity 93.9%) was administered daily in the diet to C57BL/6NCrl mice (10 of each sex per dose) at 0, 15 (males only), 50, 150, 450 or 1350 ppm (females only). The mean doses for males were 0, 3.6, 12.5, 36.7 and 109.1 mg/kg bw per day at 0, 15, 50, 150 and 450 ppm, respectively. The mean doses for females were 0, 17.6, 51.8, 156.7 and 471.2 mg/kg bw per day at 0, 50, 150, 450 and 1350 ppm, respectively. The diets were analysed for stability, homogeneity and concentration. The animals were examined for clinical signs and mortality twice a day on working days and once daily on Saturdays, Sundays and public holidays. Detailed clinical observations were performed in all animals prior to the administration period and thereafter at weekly intervals. Body weight was determined before the start of the administration period in order to randomize the animals. During the administration period, the body weight was determined on day

0 and thereafter at weekly intervals. Individual feed consumption was determined weekly over a period of 7 days and calculated as mean feed consumption in grams per animal per day. No ophthalmoscopic examination was performed. Urine parameters were not analysed in this study. At the end of the study, blood was collected for haematology and clinical chemistry measurements. All rats that died and those sacrificed at study termination were subject to gross pathological examination, and the selected tissues were collected for histological examination. The selected organs were weighed.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable (92.3–104.2% of the nominal concentrations). There were no premature deaths during the study. There were no treatment-related clinical signs of toxicity. Body weight and body weight changes were not statistically significantly different from control. However, body weight in the males at 450 ppm was decreased ($P < 0.05$) on day 14. The overall body weight gains for the males at 150 and 450 ppm were lower than for the control males. These findings in the males were considered substance related. There were no treatment-related effects on feed consumption. At the end of the study, statistically significantly decreased values for haemoglobin, haematocrit, MCV, MCH and MCHC as well as increased platelet counts were found in the peripheral blood of male mice at 150 and 450 ppm. Moreover, decreases in MCV and MCH were observed in male mice treated with 15 and 50 ppm. Although the decreases in MCV and MCH values were statistically significant and consistent with the mechanism of action of the test substance, the changes were small and not accompanied by any other effects. As such, the differences in calculated values observed at the 15 and 50 ppm levels were considered not to be biologically relevant. In female mice, haematological examinations revealed statistically significantly decreased values for haemoglobin, haematocrit, MCV and MCH at the 150, 450 and 1350 ppm levels. MCHC was decreased at 1350 ppm and platelets were increased at 450 and 1350 ppm in female mice. These changes were considered biologically relevant and adverse. In males, most of the changes in clinical chemistry parameters were minor, although a few changes were statistically significant. The values could represent normal biological variations. At 150 and 450 ppm, increased levels of serum ALT and AST activities and possibly increased blood urea levels might be related to liver pathology. There were no treatment-related clinical chemistry effects in females. The only treatment-related effect on organ weight was the increase in liver weights (absolute and relative to body weight) of males at 150 and 450 ppm and in females at 450 and 1350 ppm. Other findings were not considered relevant because of the inconsistencies, the lack of a dose–response relationship and the absence of associated histopathological findings. There were no treatment-related gross pathological findings. Substance-induced histopathological findings were observed in the liver. The findings included an increase in the occurrence and severity of a diffuse (males) or central (females) fatty change of hepatocytes, as well as increased lymphoid infiltration (both sexes), at and above 150 ppm. All other findings noted are considered spontaneous or incidental in origin and not related to treatment.

The LOAEL was 150 ppm (equal to 36.7 mg/kg bw per day in males and 51.8 mg/kg bw per day in females), based upon decreased body weight and body weight gain in males, multiple haematological changes (haemoglobin, haematocrit, MCV, MCH, MCHC and/or platelet counts) in both sexes and liver weight increases in males with centrilobular fatty change in 8 of 10 males and 1 of 10 females. The NOAEL was 50 ppm (equal to 12.5 mg/kg bw per day in males and 17.6 mg/kg bw per day in females) (Kaspers et al., 2007e).

Rats

In a 28-day toxicity study, saflufenacil (purity 94.2%) was administered daily in the diet to Wistar rats (five of each sex per dose) at 0, 50, 150, 450, 1350 or 4050 ppm (equal to 0, 4.5, 13.4, 39.2, 117 and 357 mg/kg bw per day in males and 0, 5.0, 15.0, 43.6, 130 and 376 mg/kg bw per day in females). The diets were analysed for stability, homogeneity and concentration. The animals were examined for clinical signs and mortality twice a day on working days and once daily on Saturdays,

Sundays and public holidays. Detailed clinical observations were performed in all animals prior to the administration period and thereafter at weekly intervals. Body weight was determined before the start of the administration period in order to randomize the animals. During the administration period, body weight was determined on day 0 and thereafter at weekly intervals. Individual feed consumption was determined weekly over a period of 7 days and calculated as mean feed consumption in grams per animal per day. A functional observational battery (FOB) was conducted for all rats at the end of the administration period. Motor activity was measured on the same day as the FOB. The eyes of all animals prior to the start of the administration period and the eyes of the control and high-dose animals on days 23 (males) and 22 (females) were examined for any changes using an ophthalmoscope after administration of a mydriatic. Urinalysis was conducted on samples from all study animals on day 25 (females) or 26 (males). At the end of the study, blood was collected for haematology and clinical chemistry measurements. All rats that died and those sacrificed at study termination were subject to gross pathological examination, and the selected tissues were collected for histological examination. The selected organs were weighed.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable (95.7–103.1% of the nominal concentration). There was no mortality in the study. Motor activity and FOB parameters were not affected in males or females. Several males at 450 ppm and all animals at 1350 and 4050 ppm showed dark, discoloured urine. The discoloration of the urine was considered a compensatory response to the altered porphyrin metabolism. This response is treatment related, but not adverse. Skin paleness was observed in all rats at 1350 and 4050 ppm. Urine-smeared anogenital region was observed with all males and three females at 4050 ppm and one male at 1350 ppm. There were statistically significant decreases in body weight and body weight gain in males at 1350 and 4050 ppm (76% and 75% of the control values at 1350 and 4050 ppm, respectively). Body weights and body weight changes in females were not affected. Feed consumption was reduced in males at 1350 and 4050 ppm throughout most of the study period. Isolated increases in feed consumption in females were not considered treatment related due to the lack of a clear dose–response relationship. The ophthalmoscopic examination of the eyes indicated no treatment-related findings. Dietary exposure to saflufenacil at 1350 and 4050 ppm resulted in changes in several haematological parameters in both sexes. The affected parameters were erythrocytes, haemoglobin, haematocrit, MCV, MCH, MCHC, reticulocytes, white blood cells, lymphocytes, neutrophils, anisocytosis and/or polychromasia. In males at 450 ppm, statistically significantly lower haemoglobin, MCV and MCH were also reported. Compound-related differences in serum enzyme activities were not evident at any dose level in either males or females. No treatment-related effects on serum hormone levels (triiodothyronine [T_3], thyroxine [T_4], thyroid stimulating hormone [TSH]) were found in either sex. Blood chemistry examinations revealed statistically significantly decreased total protein, albumin and globulin concentrations and increased total bilirubin levels in the males at 4050 ppm and reduced globulin concentrations as well as high total bilirubin concentrations in the serum of the males at 1350 ppm. Similar decreases in total protein, albumin and globulin levels as well as increases in total bilirubin concentrations were observed in the females at 4050 ppm. With the exception of the increase in total bilirubin values, the magnitude of changes in most of these values was small and could represent normal biological variations. Urine specimens of the males given 450, 1350 and 4050 ppm of the test compound and of the females receiving 4050 ppm were discoloured, from light yellow orange to maize yellow. In addition, urinalyses revealed statistically significantly increased urobilinogen levels in the males at 150, 450, 1350 and 4050 ppm and in the females at 4050 ppm. Slightly, but not statistically significantly, increased urinary urobilinogen concentrations were also found in the females given 1350 ppm of the test compound. No treatment-related effects were seen in the other urine parameters. The only treatment-related findings were the increases in absolute and relative spleen weights in males at 1350 and 4050 ppm and in females at 4050 ppm. The increase was associated with significant extramedullary haematopoiesis in the spleen.

The gross examinations revealed the enlarged spleens in males at 1350 and 4050 ppm and in females at 4050 ppm. Substance-induced findings were observed in the liver, spleen and bone marrow. In the liver and spleen of males at 1350 and 4050 ppm and of females at 4050 ppm, extramedullary haematopoiesis was evident. Erythroid hyperplasia was the microscopic finding in the bone marrow of males at 1350 and 4050 ppm and of females at 4050 ppm. The microscopic findings in these tissues were directly related to anaemia and porphyria. All other findings noted were considered to be spontaneous or incidental in origin and not related to treatment.

The LOAEL established in males was 450 ppm (equal to 39.2 mg/kg bw per day), based on decreased haemoglobin, MCV and MCH. Increased polychromasia and anisocytosis were also observed at this dose. The NOAEL in males was 150 ppm (equal to 13.4 mg/kg bw per day). The LOAEL established in females was 1350 ppm (equal to 130 mg/kg bw per day), based on decreased haemoglobin, haematocrit, MCV and MCH. The NOAEL in females was 450 ppm (equal to 43.6 mg/kg bw per day) (Kaspers et al., 2007b).

In a 90-day toxicity study, saflufenacil (purity 93.9%) was administered in the diet to groups of 10 male and 10 female Wistar rats of each sex per dose at dose levels of 0, 50, 150, 450 (males only), 1350 and 4050 (females only) ppm for a 3-month period. Owing to severe signs of general toxicity, the 4050 ppm dose group (females only) was terminated on day 53. The mean doses for males were 0, 3.5, 10.5, 32.3 and 94.7 mg/kg bw day at 0, 50, 150, 450 and 1350 ppm, respectively. The mean doses for females were 0, 4.3, 12.6, 110.5 and 344.7 mg/kg bw day at 0, 50, 150, 1350 and 4050 ppm, respectively. The diets were analysed for stability, homogeneity and concentration. The animals were examined for clinical signs and mortality twice a day on working days and once daily on Saturdays, Sundays and public holidays. Detailed clinical observations were performed on all animals prior to the administration period and thereafter at weekly intervals. Body weight was determined before the start of the administration period in order to randomize the animals. During the administration period, body weight was determined on day 0 and thereafter at weekly intervals. Individual feed consumption was determined weekly over a period of 7 days and calculated as mean feed consumption in grams per animal per day. A FOB was conducted for all rats at the end of the administration period. Motor activity was measured on the same day as the FOB. The eyes of all animals prior to the start of the administration period and the eyes of the control and high-dose animals (1350 ppm) on day 91 were examined for any changes using an ophthalmoscope after administration of a mydriatic. Urinalysis was conducted from all study animals at termination. At the end of the study, blood was collected for haematology and clinical chemistry measurements. All rats that died and those sacrificed at study termination were subjected to gross pathological examination, and the selected tissues were collected for histological examination. The selected organs were weighed.

The test compound was stable in the diets for 49 days at room temperature. The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the rats was acceptable (91.3–103.9% of the nominal concentrations). Owing to severe signs of general toxicity and two mortalities, the 4050 ppm dose group (females only) was terminated on day 53. At 1350 ppm, urine-smeared anogenital region was observed in two females. Two males showed skin paleness (slight) on day 91, and one showed piloerection on day 91. At 4050 ppm, body weight and body weight changes were statistically significantly decreased in females. At 1350 ppm, body weight and body weight gains of the males were decreased during the whole study period; the decrease was statistically significant during days 21–49. The body weight gain of 1350 ppm males was reduced by 14.1% of the control values at termination. In male animals of the 1350 ppm dose group, feed consumption was reduced, most of the time statistically significantly, during the whole study period (up to 10.7% on day 21). At the end of the study, statistically significantly decreased values for haemoglobin, haematocrit, MCV, MCH and MCHC and increased reticulocytes and polychromasia were found in the peripheral blood of male and female rats in the 1350 ppm dose group. In addition,

higher platelet counts and shortened prothrombin times were measured in females at this dose level. Haemoglobin, haematocrit, MCV and MCH values were also decreased in males at 450 ppm. Increased numbers of white blood cells were measured in the circulation of the male and female rats at 1350 ppm. In females, there were no treatment-related changes in the blood chemistry parameters. In males, blood chemistry examinations revealed statistically significantly increased chloride and total bilirubin concentrations in males at 1350 ppm and reduced total protein and globulin levels in males at 450 and 1350 ppm. All ophthalmoscopic findings were incidental in nature, because of their occurrence in single animals only and/or the lack of a dose–response relationship. Some males given 150, 450 and 1350 ppm of the test compound and some females receiving 1350 ppm of the test substance excreted urine that was discoloured, from maize yellow to orange. Furthermore, urinalyses revealed statistically significantly increased urobilinogen levels in males at 150, 450 and 1350 ppm and in females at 1350 ppm. Urinary bilirubin concentrations were also elevated in males given 450 and 1350 ppm of the test compound and in females exposed to 1350 ppm of the test substance. An increased number of transitional epithelial cells was also found in the urine sediments of the males at 450 and 1350 ppm, and granulated casts were detected in the urine specimens of males at 1350 ppm. In males at 1350 ppm, treatment-related effects on organ weights were noted for the spleen and heart, organs associated with treatment-related anaemia. Other statistically significant organ weight values were regarded to be secondary to the lower terminal body weight. For females at 1350 ppm, a trend towards an increase in spleen weight was regarded as a treatment-related effect. The gross macroscopic examination indicated enlarged spleens in the males of the 1350 ppm dose group. Extramedullary haematopoiesis was the main treatment-related histological finding affecting the liver of males at 1350 ppm and the spleen of males at 450 and 1350 ppm and of females at 150 and 1350 ppm. When compared with the control females, the incidence and magnitude of the effects for the females at 150 ppm were comparable, and the findings might not be considered toxicologically significant. Increased iron storage in the liver in males and females at 1350 ppm was also related to the test material. All other findings noted were considered as spontaneous or incidental in origin and were not related to treatment. No treatment-related findings were observed in the FOB parameters except for urogenital staining in two females at 1350 ppm. Statistically significantly decreased values in motor activity (intervals 2, 6 and overall) were measured in males at 1350 ppm. These findings were assessed as being related to the test article and caused by systemic toxicity and reduced body weight. A few isolated statistically significant single intervals were considered incidental.

The LOAEL established in the males was 450 ppm (equal to 32.3 mg/kg bw per day), based upon multiple clinical chemistry end-points typical of microcytic hypochromic anaemia (MHA) (decreased haemoglobin, haematocrit, MCV, MCH, total protein and globulins). Histopathological findings at this LOAEL included spleen weight increases with extramedullary haematopoiesis. The LOAEL established in the females was 1350 ppm (equal to 110.5 mg/kg bw per day), based upon multiple clinical chemistry end-points typical of MHA (decreased haemoglobin, haematocrit, MCV, MCH and MCHC). Histopathological findings at this LOAEL included spleen weight increases with extramedullary haematopoiesis.

The resulting NOAEL in both sexes was 150 ppm (equal to 10.5 mg/kg bw per day in males and 12.6 mg/kg bw per day in females) (Kaspers et al., 2007d).

Dogs

In a 4-week oral toxicity study, saflufenacil (purity 93.8%) was administered daily via gelatine capsules to purebred Beagle dogs (four of each sex per group) at 0, 30, 100 or 300 mg/kg bw per day. The dogs were examined for signs of toxicity and mortality twice a day on weekdays and once a day on Saturdays, Sundays and public holidays. Detailed clinical observations were conducted for all animals prior to the administration period and thereafter at weekly intervals. Body weight was determined before the start of the administration period in order to randomize the animals. The

weights were then determined on day 0 and weekly thereafter. The feed consumption of the animals was determined each working day, starting on day −7 (beginning of the adaptation period), and calculated as mean feed consumption in grams per animal. Ophthalmoscopic examination of the eyes was not conducted. Blood was removed from the vena cephalica antebrachii of non-anaesthetized, fasted animals for haematological and clinical chemistry measurements. Blood sampling occurred prior to dosing (on study day −14 or 13) and on study day 27. Urinalysis was conducted prior to the dosing period (study day −12 or −11) and at the end of the study (study day 23 or 24). All dogs that died and those sacrificed on schedule were subjected to gross pathological examination, and the selected tissues were collected for histological examination. The selected organs were weighed.

Treatment had no effects on mortality, body weight and body weight gain, feed consumption and feed efficiency, or gross pathology. Dark brown discoloured faeces were observed in male and female dogs in the 100 and 300 mg/kg bw per day groups. Treatment-related haematological findings were decreased erythrocyte counts, haemoglobin concentration and haematocrit values in both males and females at 300 mg/kg bw per day (Table 12). Decreased values for MCV, MCH and MCHC (males only) were also recorded in males and females at 100 and 300 mg/kg bw per day. Although the magnitude of the decreases was small and there was no clear dose–response relationship, the effects were considered biologically significant because the blood was known to be the target for saflufenacil. Alkaline phosphatase activity was higher in males and females at 300 mg/kg bw per day. Examination of porphyrin levels in the plasma, urine and faeces showed significant increases in all test groups (Table 13). The increase at 30 mg/kg bw per day, in the absence of any other adverse effects, was not considered toxicologically important. At terminal sacrifice, absolute and relative weights of the liver and spleen of males and females at 300 mg/kg bw per day were significantly higher than those of control animals. Histological examination revealed increased iron storage in the liver, extramedullary haematopoiesis in the spleen and hypertrophy of the bone marrow of male and female dogs at 300 mg/kg bw per day.

The LOAEL in both male and female dogs was 100 mg/kg bw per day, based upon MHA resulting from altered porphyrin metabolism. The NOAEL was 30 mg/kg bw per day (Kaspers et al., 2007c).

In a 90-day toxicity study, saflufenacil (purity 93.8%) was administered daily via gelatine capsules to purebred Beagle dogs (five of each sex per group) at 0, 10, 50 or 150 mg/kg bw per day. The dogs were examined for signs of toxicity and mortality twice a day on weekdays and once a day on Saturdays, Sundays and public holidays. Detailed clinical observations were conducted for all animals prior to the administration period and thereafter at weekly intervals. Body weight was determined before the start of the administration period in order to randomize the animals. The weights were then determined on day 0 and weekly thereafter. The feed consumption of the animals was determined each working day, starting on day −7 (beginning of the adaptation period), and calculated as mean feed consumption in grams per animal. Blood was removed from the vena cephalica antebrachii of non-anaesthetized, fasted animals for haematological and clinical chemistry measurements. The blood was withdrawn at three separate time points in the study: prior to the beginning of the experiment (day −14 to day −13); at the middle of the experiment (days 41–43); and at the end of the study (days 93–94). Urine was collected at days 11–12, 44–45 and 86–87 for urinalysis. All dogs were examined with an ophthalmoscope prior to and at the end of the administration period. All dogs that died and those sacrificed on schedule were subjected to gross pathological examination, and the selected tissues were collected for histological examination. The selected organs were weighed.

There were no treatment-related effects on mortality, ophthalmoscopy, urinalysis or gross pathology. Dark brown/dark red brown discoloured faeces were seen in all dogs at 150 mg/kg bw per day. This finding was likely caused by excretion of porphyrins via faeces, owing to the mode of action of saflufenacil as a PPO inhibitor. There were no other treatment-related clinical findings.

Table 12. Selected haematological values in a 28-day dog study with saflufenacil

	Mean values ± standard deviation							
	Males (n = 4 per group)				Females (n = 4 per group)			
	Dose (mg/kg bw per day)							
	0	30	100	300	0	30	100	300
Red blood cells (10^{12}/l)	6.94 ± 0.12	6.68 ± 0.45	7.32 ± 0.47	**5.75 ± 1.00**	7.00 ± 0.34	7.08 ± 0.35	7.97 ± 0.34*	**6.33 ± 0.71**
Haemoglobin (mmol/l)	9.7 ± 0.2	9.3 ± 0.7	9.3 ± 0.7	**7.4 ± 1.1**	10.3 ± 0.6	9.9 ± 0.5	10.3 ± 0.6	**8.4 ± 1.0***
Haematocrit (%)	46.6 ± 0.6	45.1 ± 3.6	45.8 ± 3.3	**36.5 ± 5.2**	49.3 ± 3.4	47.8 ± 2.3	50.0 ± 2.4	**41.1 ± 4.4***
MCV (fl)	67.2 ± 1.1	67.5 ± 2.1	**62.6 ± 0.5***	**63.6 ± 1.7***	70.3 ± 1.8	67.5 ± 2.3	**62.7 ± 1.3***	**65.0 ± 0.8***
MCH (fmol)	1.40 ± 0.03	1.40 ± 0.04	**1.27 ± 0.02***	**1.29 ± 0.03***	1.47 ± 0.03	1.40 ± 0.05	**1.30 ± 0.04***	**1.33 ± 0.02***
MCHC (mmol/l)	20.8 ± 0.20	20.7 ± 0.18	**20.3 ± 0.19***	**20.3 ± 0.38**	20.9 ± 0.24	20.8 ± 0.17	20.7 ± 0.37	20.5 ± 0.24
White blood cells (10^9/l)	11.8 ± 2.38	12.2 ± 1.27	12.9 ± 2.71	**15.2 ± 3.83**	11.6 ± 2.05	12.1 ± 2.58	12.5 ± 2.00	**17.2 ± 3.50**
Neutrophils (10^9/l)	7.02 ± 1.58	7.21 ± 0.61	8.34 ± 2.40	**9.33 ± 2.92**	6.80 ± 1.73	7.21 ± 1.85	7.38 ± 0.96	**10.39 ± 2.3**
Lymphocytes (10^9/l)	3.91 ± 0.65	4.05 ± 0.65	3.69 ± 0.27	**4.63 ± 0.90**	3.93 ± 0.40	4.06 ± 0.59	4.32 ± 0.97	**5.67 ± 0.92**
Platelets (10^9/l)	333 ± 38	332 ± 41	424 ± 17*	560 ± 102*	372 ± 44	332 ± 61	368 ± 12	552 ± 97*
Partial thromboplastin time (s)	11.9 ± 0.4	11.6 ± 0.6	10.9 ± 0.4*	10.3 ± 0.3*	11.3 ± 0.7	12.0 ± 0.5	10.5 ± 0.7	10.4 ± 0.4

From Kaspers et al. (2007c). Data taken from table 1B, pp. 72–87, 138, 142 of the study report.
* $P \leq 0.05$; **bolded** values are considered treatment related

Table 13. Porphyrin values in a 28-day dog study with saflufenacil

	Mean values ± standard deviation							
	Males (n = 4 per group)				Females (n = 4 per group)			
	Dose (mg/kg bw per day)							
	0	30	100	300	0	30	100	300
Plasma porphyrin (nmol/l)	3.9 ± 0.6	**16.5 ± 3.4***	**49.2 ± 22.9***	**121.5 ± 59.7***	4.8 ± 1.0	**16.5 ± 5.1***	**43.8 ± 26.0***	**109.8 ± 52.3***
Urinary porphyrin (µg/l)	13.1 ± 4.8	35.4 ± 21.4	**152.8 ± 14.4***	**383.8 ± 215.1***	3.1 ± 3.9	16.5 ± 11.6	**197.1 ± 107.6***	**463.8 ± 311.7***
Faecal porphyrin (µmol/kg dry faeces)	35.9 ± 28.1	**333.3 ± 151.4***	**1147.7 ± 730***	**951.7 ± 635.9***	51.5 ± 29.4	**302.0 ± 90.9***	**1247.4 ± 926.7**	**1563.6 ± 312.6***

From Kaspers et al. (2007c). Data taken from table 1B, pp. 100–101 of the study report.
* $P \leq 0.05$; **bolded** values are considered treatment related

There was no statistically significant deviation in body weight in any test group (males and females) in comparison with the control groups. Body weights of high-dose dogs were consistently lower than those of the control dogs, from day 35 to day 91 in males and for the entire dosing period in females. The lower body weights of the high-dose groups were considered treatment-induced adverse effects. Body weights and body weight gains of dogs in the 50 and 10 mg/kg bw per day groups were not affected. Feed consumption in male dogs was not affected. For the females, mean feed consumption of the high-dose dogs was lower than the control value during most of the dosing period. Mean feed efficiency data were highly variable, with large standard deviations. Consequently, there were rarely treatment-related statistically significant findings, although the high-dose dogs appeared to have lower feed efficiencies when compared with the control animals. Throughout the study period, statistically significantly decreased values for MCV, MCH and MCHC were recorded (assessed at days 41/43 and 93/94) in dogs at 150 mg/kg bw per day. It was stated in the study report that red blood cell morphology showed increased microcytosis and polychromasia at both time intervals in these dogs, and increased anisocytosis was also noted in males at 150 mg/kg bw per day on day 41 and in females at 150 mg/kg bw per day on days 43 and 94. Moreover, in males at 150 mg/kg bw per day, haemoglobin concentrations were significantly decreased on days 41 and 93, and haematocrit values were reduced on day 93. Statistically significantly increased platelet counts were observed at 150 mg/kg bw per day (males on days 41 and 93; females on day 94). However, assessment of blood clotting parameters did not show any treatment-related effects. At the end of the study, an increase in red blood cells was measured in high-dose females only. This isolated finding was considered incidental and unrelated to administered saflufenacil, because it was inconsistent with the mode of action of saflufenacil. At 50 mg/kg bw per day, significantly decreased MCH was noted on days 41 and 93, and reduced MCV in males and significantly lower values for MCV and MCH were found in females on day 94. Throughout the administration period, statistically significantly increased alkaline phosphatase activities were recorded in males at 150 mg/kg bw per day and in females at 50 and 150 mg/kg bw per day. Blood chemistry examinations revealed significantly lower total protein and albumin levels at 50 (male) and 150 (both sexes) mg/kg bw per day throughout the study. Lower albumin and bilirubin levels were also seen in males at 10 mg/kg bw per day. Total bilirubin concentrations were decreased when assessed on days 41/43 (all males and females at 50 and 150 mg/kg bw per day), but not at the end of the dosing period on days 93/94. The decreased bilirubin and albumin concentrations in males at 10 mg/kg bw per day were not considered to be treatment related because the values of both parameters were within or near the lower limit of the historical control range (Hempel, 2010). With the exception of a slight increase in relative liver weights of high-dose males (14% of control values), there were no obvious treatment-related effects on organ weights. There were no treatment-related gross pathological findings. Substance-induced microscopic findings were observed in the liver (iron storage), spleen (extramedullary haematopoiesis) and bone marrow (hypertrophy) of male and female dogs at 150 mg/kg bw per day. Iron storage in the liver and kidneys was also observed in one low-dose male and two mid-dose males. The authors considered the findings secondary to microcytic hypochromic anaemia. The defect in haem synthesis led to an excess of iron or iron-containing intermediate products, which then was intracytoplasmatically stored in liver and spleen cells. Extramedullary haematopoiesis and bone marrow hyperplasia are typical findings associated with anaemia and are considered a compensatory response.

The NOAEL was 10 mg/kg bw per day, based on lower MCV and MCH values in both sexes seen at 50 mg/kg bw per day (Kaspers et al., 2006a).

In a 1-year toxicity study, saflufenacil (purity 93.8%) was administered daily via gelatine capsules to purebred Beagle dogs (five of each sex per group) at 0, 5, 20 or 80 mg/kg bw per day. The dogs were examined for signs of toxicity and mortality twice a day on weekdays and once a day on Saturdays, Sundays and public holidays. Detailed clinical observations were conducted for all animals

prior to the administration period and thereafter at weekly intervals. Body weight was determined before the start of the administration period in order to randomize the animals. The weights were then determined on day 0 and weekly thereafter. The feed consumption of the animals was determined each working day, starting on day −7 (beginning of the adaptation period), and calculated as mean feed consumption in grams per animal. Blood was removed from the vena cephalica antebrachii of fasted anaesthetized animals. Blood sampling for haematological and clinical analyses was carried out on days −14/15, 89/90, 180/181 and 362/363. Urine was collected on days −12/13, 92/93, 183/184 and 358/359 for urinalysis. All dogs used in the study were examined with a Kowa fundus camera after administration of a mydriatic prior to and at the end of the administration period. All dogs that died and those sacrificed on schedule were subjected to gross pathological examination, and the selected tissues were collected for histological examination. The selected organs were weighed.

There were no treatment-related effects on mortality, ophthalmoscopy, urinalysis, organ weight or gross pathology. Signs of systemic toxicity were evident at 80 mg/kg bw per day only. Test substance–related findings consisted of discoloured faeces seen in four male and three female animals in the 80 mg/kg bw per day dose group. This finding was likely caused by excretion of porphyrins via faeces, due to the mode of action of the test substance as a PPO inhibitor. There were no other treatment-related findings. There was no statistically significant deviation in body weight in any test group (males and females) in comparison with the control groups. Body weights of high-dose males were consistently lower than those of the control males. The body weight gain of the high-dose males was 23% less than that of control males for the duration of the study period. The lower body weights of high-dose males were considered treatment-induced adverse effects. Body weights and body weight gains of dogs in other groups were not affected. The overall body weight gains of low- and mid-dose females were also markedly less than that of control females. The lower body weight gains of these females were not considered treatment induced, because the body weight gain of high-dose females was similar to that of the control females. Feed consumption of males was not affected. For females at 80 mg/kg bw per day, the mean feed consumption was decreased during most of the study period. The lower mean value was caused mainly by one female. This female was considered to have substance-related reduced feed consumption, along with substance-related body weight loss or retarded body weight gain and impairment of feed efficiency. The mean feed consumption over the entire administration period for this female was about 81%. In contrast, feed intake of the remaining four females at 80 mg/kg bw per day was not impaired. Throughout the study period, statistically significantly decreased values for MCV and MCH were recorded in dogs at 80 mg/kg bw per day (Table 14). The females of the 20 mg/kg bw per day dose group showed a decrease of the MCH values at the 3rd study month and a decrease of the MCV at the 6th study month. At these time points, the parameter values were isolated changes without any other haematological changes that would indicate a possible morphological dysfunction of the red blood cells in the dogs of this dose group. Furthermore, both median values were within the range of the historical controls; therefore, these deviations were not regarded as adverse effects in this dose group. At the end of the study, the high-dose males had a marginally decreased partial thromboplastin time, but other blood clotting parameters were not affected. This isolated incidence might be considered incidental.

At the 3rd, 6th and 12th study months, the alkaline phosphatase activity of the dogs at 80 mg/kg bw per day was increased (Table 15). The total protein and albumin levels were decreased at 80 mg/kg bw per day throughout the study period, statistically significantly in males as well as in females after 3 months. No treatment-related changes were found in the other blood chemistry parameters examined.

A few marginally statistically significant changes were noted. The changes were considered to be incidental and of no biological relevance because of the lack of a dose–response relationship and the absence of corresponding histopathology. There were no treatment-related gross pathological findings. Substance-induced microscopic findings were observed only in the liver. The findings were

Table 14. Selected haematological values in a 1-year dog study with saflufenacil

	Mean values ± standard deviation							
	Males (n = 5 per group)				Females (n = 5 per group)			
	Dose (mg/kg bw per day)							
	0	5	20	80	0	5	20	80
MCV (fl)								
Day 89/90	65.8 ± 1.6	66.2 ± 1.9	64.9 ± 1.3	**59.5 ± 2.1****	67.5 ± 2.0	67.1 ± 1.3	65.6 ± 1.4	**59.2 ± 1.8****
Day 180/181	66.0 ± 2.0	65.6 ± 1.6	64.3 ± 1.3	**58.5 ± 2.9****	68.2 ± 2.0	67.8 ± 1.2	66.0 ± 1.7*	**57.8 ± 2.9****
Day 362/363	64.3 ± 1.8	64.4 ± 1.3	62.4 ± 2.2	**56.2 ± 3.4****	65.9 ± 1.7	65.6 ± 1.9	63.5 ± 1.7	**55.1 ± 3.4****
MCH (fmol)								
Day 89/90	1.43 ± 0.04	1.42 ± 0.04	1.39 ± 0.03	**1.27 ± 0.06****	1.47 ± 0.04	1.45 ± 0.04	1.42 ± 0.04*	**1.27 ± 0.06****
Day 180/181	1.45 ± 0.04	1.43 ± 0.04	1.40 ± 0.03	**1.28 ± 0.08***	1.50 ± 0.04	1.49 ± 0.03	1.44 ± 0.04	**1.25 ± 0.07****
Day 362/363	1.37 ± 0.04	1.36 ± 0.03	1.32 ± 0.05	**1.19 ± 0.07****	1.41 ± 0.04	1.41 ± 0.05	1.36 ± 0.04	**1.16 ± 0.09****

From Hempel et al. (2007). Data taken from table 1B, pp. 167–192 of the study report.
MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume; * $P \leq 0.05$; ** $P \leq 0.01$; **bolded** values are considered treatment related

Table 15. Selected clinical chemistry values in a 1-year dog study with saflufenacil

	Mean values ± standard deviation							
	Males (n = 5 per group)				Females (n = 5 per group)			
	Dose (mg/kg bw per day)							
	0	5	20	80	0	5	20	80
Alkaline phosphatase (µkat/l)								
Day 89/90	1.22 ± 0.30	1.18 ± 0.38	1.75 ± 0.57	**3.04 ± 1.15****	1.38 ± 0.45	1.37 ± 0.23	1.68 ± 0.71	**3.19 ± 1.37***
Day 180/181	1.15 ± 0.30	1.37 ± 0.40	1.53 ± 0.21	**3.77 ± 1.32****	1.83 ± 0.72	1.66 ± 0.58	2.26 ± 0.68	**3.72 ± 0.95****
Day 362/363	0.95 ± 0.25	1.06 ± 0.36	1.41 ± 0.15**	**3.34 ± 1.20****	2.42 ± 1.57	1.55 ± 0.51	2.29 ± 1.74	3.50 ± 0.99
Total protein (g/l)								
Day 89/90	61.5 ± 2.14	61.9 ± 1.75	60.3 ± 1.05	**57.9 ± 1.32***	61.8 ± 2.07	60.4 ± 1.55	59.7 ± 1.39	**57.9 ± 1.93***
Day 180/181	61.3 ± 1.96	62.0 ± 1.88	59.7 ± 1.82	**56.9 ± 2.09***	61.8 ± 1.79	60.1 ± 2.29	59.0 ± 1.08	58.1 ± 2.87
Day 362/363	60.1 ± 1.63	60.7 ± 0.91	58.1 ± 1.86	**56.3 ± 1.63***	60.1 ± 1.96	59.9 ± 2.09	57.6 ± 1.24	57.0 ± 2.84
Albumin (g/l)								
Day 89/90	36.2 ± 1.54	34.9 ± 0.86	34.2 ± 1.54	**32.4 ± 1.12****	36.0 ± 1.12	36.3 ± 1.07	34.3 ± 1.41	**32.8 ± 1.84***
Day 180/181	35.7 ± 1.54	34.0 ± 1.00	34.3 ± 1.43	**31.6 ± 1.04****	35.5 ± 0.60	35.5 ± 1.59	34.4 ± 1.25	32.3 ± 2.51
Day 362/363	35.9 ± 1.61	34.7 ± 0.64	34.1 ± 0.95	**31.2 ± 0.52****	34.7 ± 0.95	35.6 ± 1.72	34.5 ± 1.24	32.8 ± 2.76

From Hempel et al. (2007). Data taken from table 1B, pp. 193–216 of the study report.
kat, katal, a Système international d'unités (SI) unit of catalytic activity; * $P \leq 0.05$; ** $P \leq 0.01$; **bolded** values are considered treatment related

slightly more pronounced for iron storage in Kupffer cells and hepatocytes of males at 20 and 80 mg/kg bw per day and of females at 80 mg/kg bw per day. This increase in iron storage was considered to be an adaptive treatment-related effect and non-adverse.

The NOAEL was 20 mg/kg bw per day, based on discoloured faeces, lower body weight in males, decreased feed consumption, lower MCV and MHC, increased serum alkaline phosphatase activity, lowered total blood protein and albumin levels, and slightly more pronounced iron storage in Kupffer cells and hepatocytes at 80 mg/kg bw per day (Hempel et al., 2007).

The overall NOAEL for the 90-day and 1-year toxicity studies in dogs was 20 mg/kg bw per day.

(b) Dermal application

Rats

In a 28-day repeated-dose dermal toxicity study, saflufenacil (purity 93.8%) was applied to the shaved skin of 10 Wistar rats of each sex per dose at 0, 100, 300 or 1000 mg/kg bw per day, 6 hours/day, 5 days/week. The test substance was suspended in an aqueous solution of 0.5% carboxymethylcellulose. The test substance was administered uniformly to the clipped dorsal skin (dorsal and dorsolateral parts of the trunk; at least 10% of the body surface area) using 3 ml syringes. The administration volume was 4 ml/kg bw, based on the latest individual body weight determination. The skin was covered for 6 hours after application using a semi-occlusive dressing, consisting of four layers of porous gauze dressing and held in place with an elastic top dressing. After removal of the dressing, the treated skin was washed with lukewarm water. Control animals received only the vehicle. Dosing preparations were analysed for stability, homogeneity and concentration. Animals were checked for mortality and clinical signs of toxicity twice daily on working days and once daily on Saturdays, Sundays and public holidays. Body weights were recorded before the start of the study (day 0) and thereafter at weekly intervals. Individual feed consumption was determined weekly. A FOB was performed in all animals at the end of the study. Motor activity was measured with all animals on the same day on which the FOB was performed. A blood sample was collected for haematological and clinical chemistry parameters at the end of the study. Urinalysis was conducted at the end of the study. The eyes of all animals prior to the start of the administration period and the eyes of the control and high-dose animals on day 27/28 (females/males) were examined for any changes using an ophthalmoscope after administration of a mydriatic. All rats that died and those sacrificed at study termination were subject to gross pathological examination, and the selected tissues from the control and high-dose rats were collected for histological examination. The selected organs were weighed.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable. All rats survived to study termination. There were no treatment-related effects on clinical signs, feed consumption and feed efficiency, skin reaction, ophthalmoscopy, FOB, motor activity, clinical chemistry, gross pathology or histopathology. The only treatment-related finding was a slight decrease in haemoglobin concentration in males at 1000 mg/kg bw per day (97% of the controls). The marginal change in the haematological parameter was not considered adverse by the Meeting because the magnitude of the change was small and statistical significance was not achieved. Urinalyses revealed significantly increased urobilinogen levels in males at 1000 mg/kg bw per day and in females at 300 and 1000 mg/kg bw per day. Moreover, urine specimens of the high-dose males were discoloured (light orange), which was likely related to increased urinary excretion of porphyrins. The changes in urine parameters were considered to be compensatory responses to the altered porphyrin metabolism. As these changes in urinary parameters were not associated with other pathological alterations, they were not considered adverse.

The NOAEL was 1000 mg/kg bw per day, the highest dose tested. The study authors concluded that, under the conditions of the study, the NOAEL was 300 mg/kg bw per day for males due to a mild

anaemic process, based on the mode of action of the test substance, and 1000 mg/kg bw per day for females (Kaspers et al., 2006b).

(c) Exposure by inhalation

No studies were submitted.

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a carcinogenicity study, saflufenacil (purity 93.8%) was administered daily in the diet to groups of C57BL/6NCrl mice (50 of each sex per group) at 0, 1 (males only), 5, 25, 75 or 150 (females only) ppm for an 18-month period. The mean doses for males were 0, 0.2, 0.9, 4.6 and 13.8 mg/kg bw per day at 0, 1, 5, 25 and 75 ppm, respectively. The mean doses for females were 0, 1.2, 6.4, 18.9 and 38.1 mg/kg bw per day at 0, 5, 25, 75 and 150 ppm, respectively. In satellite groups of 10 mice of each sex per group, saflufenacil was administered daily in the diet at 0, 75 (males only) and 150 (females only) ppm (equal to 0 and 14.2 mg/kg bw per day for males and 0 and 39.0 mg/kg bw per day for females) over a period of 10 months. The diets were analysed for stability, homogeneity and concentration. The animals were examined for clinical signs and mortality twice a day on working days and once daily on Saturdays, Sundays and public holidays. Detailed clinical observations were performed on all animals prior to the administration period and thereafter at weekly intervals. Body weight was determined before the start of the administration period in order to randomize the animals. The weights were then determined on day 0, weekly during the first 13 weeks, at 4-week intervals thereafter and prior to the start of necropsy. Feed consumption was determined once a week over a period of 7 days during the first 13 weeks of the administration period, at 4-week intervals thereafter and prior to the start of necropsy. The values were calculated as feed consumption in grams of feed per mouse per day. Haematology was assessed in satellite mice only and on day 303/304. No clinical parameters were assessed. Urine parameters were not assessed. The eyes were not examined. Faeces were collected overnight from all satellite animals. At scheduled sacrifice, parts of the liver of the satellite mice were removed. The collected faecal and liver samples were analysed for porphyrin levels. All mice that died and those sacrificed at study termination were subject to gross pathological examination, and the selected tissues were collected for histological examination from the controls and the high-dose animals. In addition, gross lesions, liver, bone marrow (femur) and spleen from all mice were subjected to histopathological examination. The selected organs were weighed.

The test substance was stable in the diets for a period of 49 days. The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the mice was acceptable (91.2–110.3% of the nominal). There were no treatment-related effects on clinical signs, mortality, body weight and body weight gain, feed consumption and feed efficiency, gross pathology or organ weights. As a result of the high mortality observed in test and control males (Table 16), all surviving males were sacrificed on study days 527 and 528 (approximately 20 days before the originally scheduled sacrifice).

In the 10th month of the study, statistically significantly reduced red blood cell counts, haemoglobin concentrations and haematocrit values were recorded for the males at 75 ppm and for the females at 150 ppm in the satellite group (Table 17). The red blood cell indices (MCH, MCHC, MCV) as well as the reticulocyte counts were not changed compared with the controls. Also, the total white blood cell counts, as well as the differential blood cell counts, showed no significant differences between the treated mice and the controls of both sexes. There were no treatment-related findings in the differential blood cell count and the red or white blood cell morphology of the high-dose mice (males 75 ppm; females 150 ppm) at 10 months of the treatment and at termination in the main study groups.

Table 16. Mortality rates of mice in the carcinogenicity study with saflufenacil

Dietary concentration (ppm)	Mortality (%)	
	Males	Females
0 (satellite)	0	0
0	72	58
1	62	—
5	72	56
25	60	68
75 (satellite)	0	—
75	56	60
150 (satellite)	—	10
150	—	58

From Kamp et al. (2007)

Table 17. Selected haematological values from satellite mice, day 304, in the carcinogenicity study with saflufenacil

	Mean values ± standard deviation			
	Males		Females	
	Dietary concentration (ppm)			
	0	75	0	150
	n = 10	n = 10	n = 10	n = 9
Red blood cells (10^{12}/l)	8.94 ± 0.26	**8.33 ± 0.23****	8.61 ± 0.40	**8.01 ± 0.73***
Haemoglobin (mmol/l)	8.1 ± 0.3	**7.4 ± 0.3****	7.8 ± 0.4	**7.3 ± 0.6***
Haematocrit (%)	40.3 ± 1.4	**37.1 ± 1.5****	38.4 ± 1.5	**35.9 ± 2.7***

From Kamp et al. (2007); data taken from table 1B, pp. 164–165 of the study report.
* $P \leq 0.05$; ** $P \leq 0.01$; **bolded** values are considered treatment related

The mean porphyrin values in the faeces and liver were statistically significantly increased in the treated animals, although there were large interindividual variations (Table 18).

The only notable microscopic finding was in the liver. There was a significant increase in lipogenic pigment in the liver of males at 25 and 75 ppm and in females at 75 and 150 ppm (Table 19). Although the deposition of lipogenic pigment was considered to be treatment related, it is a common finding in older mice and might be accelerated by treatment with certain chemicals. As this finding was not associated with other histological changes in the liver and was not considered a precancerous lesion, the finding was not considered adverse. Enlarged nuclei of variable size (karyomegaly) occurred in centrilobular hepatocytes, especially in treated males. Karyomegaly was a common age-related finding in mice, the biological significance of which is unclear. As this finding was not associated with other adverse effects (e.g. hyperplasia) and was not considered to be a carcinogenic precursor, the finding was not considered adverse. All other non-neoplastic findings (including those in the decedents) either occurred singly or were equally distributed among control and test groups. They were considered to be incidental or spontaneous in origin and unrelated to treatment.

All neoplastic findings either occurred singly or were equally distributed among control and test groups. They were considered spontaneous or incidental in nature and unrelated to treatment. Under the conditions of the study, saflufenacil demonstrated no carcinogenic potential up to the highest dose levels tested.

Table 18. Faecal and liver porphyrin values from satellite mice, day 304, in the carcinogenicity study with saflufenacil

	Mean values ± standard deviation			
	Males		Females	
	Dietary concentration (ppm)			
	0	75	0	150
	n = 10	*n* = 10	*n* = 10	*n* = 9
Faecal total porphyrin (µmol/kg dry faeces)	15.2 ± 5.2	**432 ± 75****	9.2 ± 8.8	**463 ± 273***
Liver total porphyrin (pmol/g protein)	273 ± 104	**8146 ± 5546****	139 ± 46	**14 332 ± 14 159****

From Kamp et al. (2007); data taken from table 1B, pp. 170–171 of the study report.
* $P \leq 0.05$; ** $P \leq 0.01$; **bolded** values are considered treatment related

Table 19. Selected microscopic findings in the carcinogenicity study with saflufenacil

	No. of mice affected									
	Males (*n* = 50 per group)					Females (*n* = 50 per group)				
	Dietary concentration (ppm)									
	0	1	5	25	75	0	5	25	75	150
Liver, focal lipogenic pigment	0	0	0	9**	37**	4	2	4	15**	21**
Liver, karyomegaly	2	0	0	6	16**	1	0	0	1	0

From Kamp et al. (2007). Data taken from table 1C, pp. 193–248 of the study report.
** $P \leq 0.01$; **bolded** values are considered treatment related

The NOAELs for systemic toxicity were 25 ppm (equal to 4.6 mg/kg bw per day) in males and 75 ppm (equal to 18.9 mg/kg bw per day) in females, based on anaemia and porphyria observed in the satellite group at 75 ppm (equal to 13.8 mg/kg bw per day) in males and 150 ppm (equal to 38.1 mg/kg bw per day) in females. Saflufenacil was not carcinogenic at the dose levels tested. The NOAELs for carcinogenicity were the highest doses tested (75 ppm, equal to 13.8 mg/kg bw per day, for males and 150 ppm, equal to 38.1 mg/kg bw per day, for females) (Kamp et al., 2007).

Rats

In a combined 2-year toxicity and carcinogenicity study, saflufenacil (purity 93.8%) was administered in the diet to Wistar rats (50 of each sex per group; satellite groups of 10 of each sex per group) at 0, 20, 100, 250 (males only), 500 or 1000 (females only) ppm for 24 months. The satellite groups were dosed for 12 months. The mean doses for males were 0, 0.9, 4.8, 12.0 and 24.2 mg/kg bw per day at 0, 20, 100, 250 and 500 ppm, respectively. The mean doses for females were 0, 1.3, 6.2, 31.4 and 63.0 mg/kg bw per day at 0, 20, 100, 500 and 1000 ppm, respectively. The diets were analysed for stability, homogeneity and concentration. The animals were examined for clinical signs and mortality twice a day on working days and once daily on Saturdays, Sundays and public holidays. Detailed clinical observations were performed on all animals prior to the administration period and at weekly intervals thereafter. Body weight was determined before the start of the administration period in order to randomize the animals. The weights were then determined on day 0, weekly during the first 13 weeks, at 4-week intervals thereafter and prior to the start of necropsy. Feed consumption was determined once a week over a period of 7 days during the first 13 weeks of the administration period, at 4-week intervals thereafter and prior to the start of necropsy. The values were calculated as feed consumption in grams feed per rat per day. Prior to the administration period, the eyes of all rats were

examined with an ophthalmoscope. The eyes of the control and high-dose rats were also examined on day 353/354. Haematological and clinical chemistry parameters were examined on days 92, 176 and 358. Urinalysis was conducted on days 88, 178/179 and 360/361. All rats that died and those sacrificed at study termination were subject to gross pathological examination, and selected tissues were collected for histological examination. For the rats in the two lowest-dose groups (i.e. 20 and 100 ppm), only the liver, lungs, bone marrow, spleen, kidneys and gross lesions were processed for histopathological examination. Selected organs were weighed.

The test substance was stable in the diets for a period of 49 days. The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the rats was acceptable (91.4–109.6% of the nominal). Compared with the control group (6/60), higher incidences of urine-smeared anogenital region were observed in females at 500 ppm (9/60) and 1000 ppm (21/60). These findings also occurred at earlier times than those seen in control rats and were assessed as treatment related. There was no excessive mortality in any group (Table 20).

Body weight and body weight gain of the females were not affected. In males, mean body weight at 500 ppm was slightly decreased during the first 10–30 weeks of dosing. The decreases were rarely higher than 5% of the control values. Weekly body weight changes varied considerably, but did not show a clear treatment-related adverse effect. The decreases in body weight and body weight gains in the 500 ppm males are considered treatment-related effects. There were no treatment-related effects on feed consumption or feed efficiency. All ophthalmoscopic findings were considered to be incidental in nature, because they were observed only in single animals and/or occurred without a dose–response relationship. Treatment-induced haematological effects were lower haemoglobin concentration, haematocrit, MCV and MCH in males at 500 ppm and females at 1000 ppm at all time points assessed. In addition, decreases in haemoglobin and haematocrit values were also recorded in females at 500 ppm on day 176. No significant changes in the differential blood count were noted in any dose group. There were no treatment-related adverse effects on serum enzyme activities. The only noted findings in clinical chemistry parameters were the slight decreases in protein (on day 176 at 250 and 500 ppm) and albumin (on day 176 at 500 ppm) levels in males. Significant changes in these clinical chemistry parameters, in any dose group, were not noted after 1 year of treatment. The marginally statistically significant findings might be transient in nature, although an association with dietary exposure to saflufenacil could not be ruled out, because lower protein levels were recorded in other toxicity studies after oral administration of saflufenacil. No changes in any clinical chemistry parameters were noted in females of any dose group at any time point. Treatment-related urinalysis findings were generally higher urobilinogen values in males (250 and 500 ppm) and females (500 and 1000 ppm). The authors considered that the increase in urinary urobilinogen represented heightened porphyrin metabolism in the liver, resulting in higher accumulation in the circulation, which was effectively excreted in the urine and not considered as an adverse effect. There were no significant absolute organ weight deviations. In males, the mean relative weights of epididymides were significantly increased at 250 (+10.2%, $P < 0.05$) and 500 ppm (+10.7%, $P < 0.01$). The increased relative weights of epididymides were related to the slightly decreased terminal body weights in these animals (−7.6% and −9.4%, respectively). Statistically significant changes in organ weights in males were lower absolute and relative spleen and kidney weights at 500 ppm and higher absolute brain weights at 100, 250 and 500 ppm. The increase of the mean absolute brain weights was not dose related. The mean relative brain weights did not show significant changes; in addition, there were no histopathological correlates. Therefore, the increased brain weights were considered incidental. The decreased absolute and relative kidney and spleen weights did not show a clear dose–response relationship, and there were no pathological findings that explain the weight changes. Additionally, saflufenacil, at toxic doses, causes increased, rather than decreased, spleen weights as a compensatory response to anaemia. Therefore, the spleen weight decreases were not considered a treatment-related finding. In females, no significant weight deviations were recorded. There were no treatment-related gross necropsy findings in either the satellite or the main study groups. All non-neoplastic findings in the

Table 20. Mortality rate in a carcinogenicity study in rats

Test group	Dietary concentration (ppm)	Mortality (%)	
		Males	Females
0	0	22	30
1	20	25	28
2	100	22	33
3	250	30	—
4	500	17	23
5	1000	—	22

From Kaspers et al. (2007f)

satellite or the main study groups either occurred singly or were biologically equally distributed over the control group and the treatment groups. They were considered to be incidental or spontaneous in origin and without any relation to treatment. All neoplastic findings in the satellite or the main study groups either occurred singly or were biologically equally distributed over the control group and the treatment groups. As such, all neoplastic lesions were considered spontaneous or incidental in nature and not related to treatment. Saflufenacil was not considered oncogenic in rats. There were small differences in the incidence of uterine and thyroid tumours between control and high-dose animals. The incidence of uterine adenocarcinoma was higher in high-dose females (10/50) than in the control females (5/50). The incidence of thyroid C-cell adenoma was higher in high-dose males (10/50) than in control males (3/49). However, both tumour types in the high-dose animals were within the historical control range. The historical control values for uterine adenocarcinoma in six studies (n = 50 per study) were 7, 1, 1, 15, 12 and 9. The historical control values for thyroid C-cell adenoma in six studies (n = 50 per study) were 10, 9, 10, 7, 11 and 11. Thus, the observation of a slight increase in uterine adenocarcinoma and thyroid C-cell adenoma in high-dose rats in the present study was not considered treatment related. In addition, the incidence of the potentially preneoplastic focal/multifocal C-cell hyperplasia is very similar for males at 500 ppm (6/50) compared with controls (6/49). Therefore, for the reasons given above, the increased incidence (10/50) (20%) of C-cell adenoma for males receiving saflufenacil at 500 ppm (highest concentration tested), compared with the control incidence of 3/49 (approximately 6%), should not be considered treatment related.

The NOAELs for oncogenicity were the highest dose tested: for males, 500 ppm (equal to 24.2 mg/kg bw per day), and for females, 1000 ppm (equal to 63.0 mg/kg bw per day). The NOAELs for systemic toxicity were 250 and 100 ppm for the male and female rats (equal to 12.0 and 6.2 mg/kg bw per day for males and females, respectively), based on decreased body weight and body weight gain in males, anogenital region smeared with urine in females, and lower haemoglobin concentration, haematocrit, MCV and MCH in males and females at 500 ppm (equal to 24.2 and 31.4 mg/kg bw per day for males and females, respectively) (Kaspers et al., 2007f).

2.4 Genotoxicity

A battery of studies of mutagenicity with saflufenacil was conducted to assess potential for inducing gene mutation, chromosomal aberration and unscheduled deoxyribonucleic acid (DNA) synthesis. The study results (summarized in Table 21) were negative except for positive results in the in vitro mammalian clastogenicity assay in the presence of a metabolic activation system. In this study, the test substance saflufenacil led to a statistically significant increase in the number of structural chromosomal aberrations, including and excluding gaps, after the addition of a metabolizing system in two experiments performed independently of each other after an exposure time of 4 hours

Table 21. Mutagenicity studies with saflufenacil

End-point	Test object	Concentration	Purity (%)	Results	Reference
In vitro					
Bacterial reverse mutation (Ames test)	*Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* WP2*uvrA*	0, 55, 174, 550, 1740 or 5500 µg/plate (with and without S9 mix)	93.8	Negative	Engelhardt & Leibold (2005a)
Bacterial reverse mutation[a] (Ames test)	*S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* WP2*uvrA*	0, 20, 100, 500, 2500 or 5500 µg/plate (with and without S9 mix)	99.0	Negative	Engelhardt & Leibold (2005e)
Mammalian clastogenicity	Chinese hamster lung (V79) cells	1st: 0, 156.25, 312.5, 625, 1250, 2500 or 5000 µg/ml 2nd: 0, 250, 500, 1000, 2000 or 4000 µg/ml (with and without S9 mix)	93.8	−S9: Negative +S9: Positive	Engelhardt & Leibold (2005f)
Forward mutation assay in mammalian cells (HPRT test)	Chinese hamster ovary cells	0, 312.5, 625, 1250, 2500 or 5000 µg/ml (with and without S9 mix)	93.8	Negative	Engelhardt & Leibold (2005c)
In vivo					
Mouse micronucleus	NMRI mice, male	0, 500, 1000 or 2000 mg/kg bw (oral gavage in 0.5% carboxymethylcellulose) Sampling time: 24 h for 500 and 1000 mg/kg bw group; 48 h for 2000 mg/kg bw group	93.8	Negative	Engelhardt & Leibold (2005b)
Unscheduled DNA synthesis	Wistar rats, male	0, 1000 or 2000 mg/kg bw (oral gavage in 0.5% carboxymethylcellulose) Sampling times: 3 h, 14 h	93.8	Negative	Engelhardt & Leibold (2005d)

S9, 9000 × *g* rat liver supernatant
[a] Saflufenacil anhydrate form.

and a sampling time of 28 hours. Saflufenacil did not induce an increase in chromosomally damaged cells, including and excluding gaps, at other time points with metabolic activation or under any treatment conditions without addition of a metabolic activation system. However, it was negative for clastogenicity in the mouse micronucleus assay. Overall, saflufenacil is not likely to be genotoxic.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

In a two-generation study, saflufenacil (purity 93.8%) was administered in the diet to groups of 25 male and 25 female healthy young Wistar rats (F_0 parental generation) at 0, 5, 15 or 50 mg/kg bw per day. At least 75 days after the beginning of treatment, F_0 animals were mated to produce a litter (F_1). Mating pairs were from the same dose group, and F_1 animals selected for breeding were continued in the same dose group as their parents. Groups of 25 males and 25 females, selected from

F_1 pups to become the F_1 parental generation, were offered diets containing the test substance at 0, 5, 15 or 50 mg/kg bw per day post-weaning, and the breeding programme was repeated to produce an F_2 litter. The study was terminated with the terminal sacrifice of the F_2 weanlings and F_1 parental animals. Test diets containing saflufenacil were adjusted regularly to obtain the desired doses throughout the study. Test diets were analysed for stability, homogeneity and concentration. The rats were observed for clinical signs and mortality twice daily on weekdays and once on Saturdays, Sundays and public holidays. In general, body weights of the male and female parents were determined on the first day of the pre-mating period and then once a week. Feed consumption was determined once a week (each time for a period of at least 6 days) for the F_0 and F_1 parents. Feed consumption of the females during pregnancy was determined weekly for days 0–7, 7–14 and 14–20 post-coitus and during lactation for days 1–4, 4–7 and 7–14 postpartum. Estrous cycle length and normality were evaluated for females. Preputial separation and sperm parameters were evaluated for males. Blood samples were withdrawn from all non-fasted F_0 and F_1 parents before sacrifice. Blood samples were taken on lactation days 4 (post-culling) and 21 from F_1 and F_2 pups (10 of each sex per group). All surviving parental males and females were subjected to gross pathological examination (external and internal examinations, including the cervical, thoracic and abdominal viscera). The culled F_1 and F_2 offspring were sacrificed on lactation day 4. The F_2 offspring carried through the lactation period were sacrificed at day 21 postpartum. The pups were examined externally and eviscerated, and their organs were assessed macroscopically.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the rats was acceptable (89.7–107.7% of the nominal values). There were no deaths. No treatment-related clinical signs of toxicity were observed in males or females in any dose group. During pre-mating, mean body weights and body weight gains of the F_0 parents of all test groups were generally comparable to those of the controls. Mean body weights of F_0 females of all test groups were comparable to those of the controls during gestation, lactation and post-weaning periods. However, mean body weight gain in high-dose F_0 females was statistically significantly below the control value on gestation days 7–20 and the beginning of lactation (days 1–4). Thereafter, body weight gains of high-dose females recovered. Mean body weights of high-dose F_1 males were statistically significantly lower (up to approximately 10%) from week 5 onwards until the end of the study. For the entire dosing period (weeks 0–15), mean body weight gain of high-dose males was about 11% below the concurrent control value. Mean body weights and body weight gain of F_1 females were comparable to those of the control group during the pre-mating period. Mean body weights of F_1 parental females in the 50 mg/kg bw per day dose group were statistically significantly lower (approximately 6%) than those of controls on gestation day 20. Mean body weight gain was statistically significantly decreased in these females on days 14–20 post-coitus (about 21%) and days 0–20 post-coitus (about 12%) and was regarded as a treatment-related effect. At the beginning of lactation (days 1–4 postpartum), the high-dose F_1 females still gained significantly less weight (−49%) than the concurrent controls, but recovered afterwards. Feed consumption of the F_0 male rats of all test groups was generally comparable to that of the controls throughout the entire study. Feed consumption of the F_0 females of the 50 mg/kg bw per day dose group was also comparable to that of the controls during premating, gestation and post-weaning. However, during the entire lactation period, the feed consumption of the high-dose F_0 females was statistically significantly below that of controls (as high as about −19%) on days 1–4. Feed intake of high-dose F_1 males was consistently lower than that of the control males during weeks 4–15 of the pre-mating period. Feed consumption of high-dose F_1 females was statistically significantly decreased during pre-mating weeks 4–7 and during lactation days 1–14. These were considered treatment related. The body weights and feed consumption of F_0 parental and F_1 parental animals at the middle and low doses were comparable to those of concurrent controls. After dietary exposure to saflufenacil for about 17 weeks for the males and about 19 weeks for the females (including pregnancy), the rats showed signs of MHA. Haemoglobin concentrations and other indices of the

red blood cell (haematocrit, MCV, MCH and/or MCHC) were decreased in both sexes at 50 mg/kg bw per day. The decreases in haemoglobin, haematocrit, red blood cells, MCH and/or MCV in F_0 and F_1 males and in haemoglobin in F_0 females at 15 mg/kg bw per day were marginal (≤ 5%) and were not considered toxicologically significant. The effects of dietary exposure to saflufenacil on clinical parameters were highly consistent in F_0 and F_1 parents. When compared with the concurrent controls, serum protein levels were marginally (3–6%) but statistically significantly lower in high-dose F_0 and F_1 rats. In high-dose males, this decrease was due to both protein fractions (albumin and globulin). The triglyceride values were also decreased in high-dose males of both generations. At 15 mg/kg bw per day, the decreases in total protein and albumin levels in F_1 males were marginal and were not considered toxicologically significant. Evaluation of the estrous cycle data for 3 weeks prior to mating for the F_1 and F_2 litters revealed very regular cycles (mean of 4.0–4.8 days) in the F_0 and F_1 females of all groups. There were no treatment-related effects noted for the sperm parameters examined at or after the sacrifice of the F_0 and F_1 males. No treatment-related effects on the mating and fertility of the F_0 and F_1 males (96–100%) were observed. All sperm-positive F_0 females delivered pups or had implants in utero. Thus, the fertility index was 100% for all groups. The mean duration of gestation of F_0 females was similar among all groups (21.8–22.2 days) and was within the historical control range of 21.5–22.3 days. The gestation indices of the F_0 females were comparable among all groups. The mean number of implantation sites of the F_0 females was comparable among all dose groups (12.2, 12.3, 12.2 and 11.8 implants per dam at 0, 5, 15 and 50 mg/kg bw per day, respectively). There were no statistically significant differences in post-implantation loss among dose groups. However, the high-dose post-implantation loss of 14.6% was above the historical control range of the test facility (2.5–9.0%). As no such effects were seen in the subsequent F_1 generation, this finding was regarded as incidental and not treatment related. The number of liveborn pups was, however, lowest, and the number of stillborn pups and pups that died after birth was highest, at 50 mg/kg bw per day in both F_0 and F_1 generations, and the changes were both statistically significant (Table 22). The statistically significant decreases in the liveborn pups and the live birth index, as well as the significant increase in stillborn pups and pups that died after birth, in the high-dose group were considered treatment related.

Organ weight changes of the F_0 and F_1 male rats are shown in Table 23. The weights of several organs from the high-dose males were statistically significantly different from the concurrent control values. The findings were mostly attributed to the significant reduction in terminal body weight of these rats. The increased absolute and relative spleen weights in high-dose males were consistent with the findings in the F_0 males and were probably related to dietary exposure to saflufenacil. The increased absolute and relative mean thyroid weights and the increased relative weights of ovaries in low-dose females (Table 24) as well as the decreased relative liver weight in mid-dose males are considered incidental.

All gross lesions observed in F_0 and F_1 test animals occurred singularly. They are considered to be spontaneous in origin and are not related to treatment. The F_0 female rat that was not pregnant did not show relevant gross lesions. The F_0 male mating partner showed a severe reduction in sizes of testes and epididymides. The non-pregnant F_1 females and their F_1 male mating partners did not show gross lesions explaining the infertility. All histopathological findings noted either were single observations or were similar in distribution pattern and severity in control and test rats. All findings were considered incidental and unrelated to dietary exposure to saflufenacil. The non-pregnant low-dose F_0 female did not show histopathological findings explaining the infertility. The male mating partner showed, corresponding to gross lesions, a moderate diffuse tubular degeneration in the left testicle resulting in epididymis aspermia, causing the impaired fertility. The findings in the testes and epididymides were considered incidental. For two of the non-pregnant F_1 females, a dilatation of uterus horns was observed. One of the mating partners showed a moderate chronic inflammation of the prostate. These findings did not explain the infertility and were considered incidental. The other non-pregnant F_1 female and two of the male mating partners did not show histopathological findings explaining the infertility.

Table 22. Summary of selected F_0 and F_1 female reproductive data in a two-generation study in rats with saflufenacil

	Mean values ± standard deviation							
	F_0 (n = 25 per group)				F_1 (n = 25 per group)			
	Dose (mg/kg bw per day)							
	0	5	15	50	0	5	15	50
Number mated	25	24	25	25	25	25	25	25
Number pregnant	25	24	25	25	24	24	25	24
Number with liveborn	25	24	25	24	24	24	25	24
Number with stillborn pups	2	1	3	**7**	4	0	1	**9**
Duration of gestation (days)	21.8 ± 0.5	21.9 ± 0.45	22.0 ± 0.2	22.2 ± 0.51**	21.7 ± 0.46	22.0 ± 0.42	21.9 ± 0.33	22.0 ± 0.51
Post-implantation loss								
- total	21	21	15	35	12	16	13	21
- %	6.8 ± 8.6	7.1 ± 8.1	5.0 ± 7.0	**14.6 ± 20.7**	5.0 ± 8.66	6.7 ± 8.25	4.6 ± 6.02	8.3 ± 10.3
- per dam	0.8 ± 1.03	0.9 ± 0.99	0.6 ± 0.87	**1.4 ± 1.32**	0.5 ± 0.78	0.7 ± 0.82	0.5 ± 0.65	0.9 ± 1.12
Pups delivered								
- total	285	273	291	259	247	254	279	227
- per dam	11.4 ± 1.96	11.4 ± 2.08	11.6 ± 2.04	10.8 ± 2.02	10.3 ± 2.22	10.6 ± 2.22	11.2 ± 1.75	9.5 ± 2.32
- liveborn								
- total	283	272	288	**240****	243	254	278	**211****
- per dam	11.3	11.3	11.5	**10**	10.1	10.6	11.1	**8.8**
- stillborn								
- total	2	1	3	**19****	4	0	1	**16****
- per dam	0.08	0.003	0.12	**0.79**	0.17	0	0.04	**0.67**
- died								
- total	0	5	0	**9****	1	1	1	**12****
- per dam	0	0.21	0	**0.38**	0.04	0.04	0.04	**0.5**

From Schneider et al. (2007a). Data taken from table 1A, pp. 159–161, 205–207 of the study report.
** $P \leq 0.01$; **bolded** values are considered treatment related

Table 23. Selected organ weights of F_0 and F_1 male rats in a two-generation study with saflufenacil

Organ	Mean weights ± standard deviation			
	Dose (mg/kg bw per day)			
	0	5	15	50
F_0 (*n* = 25 per group)				
Body weight (g)	*365.2 ± 32.8*	*370.2 ± 29.9*	*369.1 ± 31.5*	*351.1 ± 40.0*
Adrenal				
- absolute (mg)	61.1 ± 8.13	55.1 ± 6.27**	55.8 ± 6.16*	56.9 ± 5.08*
- relative (/bw)	0.017 ± 0.002	0.015 ± 0.002**	0.015 ± 0.002*	0.016 ± 0.002
Spleen				
- absolute (g)	0.628 ± 0.119	0.614 ± 0.091	0.601 ± 0.063	**0.703 ± 0.112***
- relative (/bw)	0.172 ± 0.031	0.166 ± 0.022	0.164 ± 0.019	**0.201 ± 0.032****
Epididymides				
- absolute (g)	1.122 ± 0.076	1.125 ± 0.156	1.126 ± 0.103	1.163 ± 0.103
- relative (/bw)	0.309 ± 0.024	0.305 ± 0.039	0.307 ± 0.032	0.334 ± 0.031**
Seminal vesicle				
- absolute (g)	0.943 ± 0.174	1.028 ± 0.200	1.050 ± 0.115	1.041 ± 0.176
- relative (/bw)	0.258 ± 0.042	0.279 ± 0.054	0.287 ± 0.043*	0.297 ± 0.044*
F_1 (*n* = 25 per group)				
Body weight (g)	*372.6 ± 31.1*	*366.4 ± 42.0*	*373.1 ± 35.5*	***331.7 ± 32.9****
Brain				
- absolute (g)	2.059 ± 0.103	2.024 ± 0.092	2.022 ± 0.101	**1.977 ± 0.084****
- relative (/bw)	0.556 ± 0.048	0.558 ± 0.057	0.546 ± 0.05	**0.6 ± 0.043****
Adrenal				
- absolute (mg)	64.6 ± 11.1	58.7 ± 8.11	59.5 ± 6.77	59.0 ± 6.45
- relative (/bw)	0.017 ± 0.002	0.016 ± 0.002*	0.016 ± 0.002	0.018 ± 0.002
Spleen				
- absolute (g)	0.619 ± 0.1	0.608 ± 0.105	0.609 ± 0.065	**0.945 ± 1.0****
- relative (/bw)	0.167 ± 0.029	0.167 ± 0.028	0.164 ± 0.019	**0.28 ± 0.264****
Liver				
- absolute (g)	8.602 ± 0.855	8.176 ± 1.047	8.300 ± 1.018	7.581 ± 0.831**
- relative (/bw)	2.308 ± 0.111	2.231 ± 0.124	2.221 ± 0.109*	2.285 ± 0.106
Kidneys				
- absolute (g)	2.361 ± 0.255	2.248 ± 0.222	2.275 ± 0.239	2.093 ± 0.204**
- relative (/bw)	0.634 ± 0.051	0.616 ± 0.048	0.61 ± 0.04	0.633 ± 0.051
Pituitary gland				
- absolute (mg)	9.12 ± 2.068	9.08 ± 1.913	9.2 ± 1.893	8.8 ± 1.633
- relative (/bw)	0.002 ± 0.01	0.002 ± 0.0	0.002 ± 0.01	0.003 ± 0.0
Testes				
- absolute (g)	3.697 ± 0.344	3.67 ± 0.332	3.65 ± 0.275	3.512 ± 0.284
- relative (/bw)	0.998 ± 0.11	1.01 ± 0.112	0.984 ± 0.098	1.063 ± 0.073**
Epididymides				
- absolute (g)	1.11 ± 0.115	1.1 ± 0.097	1.082 ± 0.068	1.073 ± 0.118
- relative (/bw)	0.299 ± 0.036	0.303 ± 0.031	0.292 ± 0.027	0.323 ± 0.029**
Cauda epididymides				
- absolute (g)	0.422 ± 0.039	0.418 ± 0.041	0.423 ± 0.039	0.419 ± 0.042
- relative (/bw)	0.114 ± 0.014	0.115 ± 0.012	0.114 ± 0.012	0.127 ± 0.01**
Seminal vesicle				
- absolute (g)	1.152 ± 0.185	1.117 ± 0.186	1.113 ± 0.198	1.117 ± 0.223
- relative (/bw)	0.31 ± 0.051	0.306 ± 0.047	0.298 ± 0.042	0.337 ± 0.061

From Schneider et al. (2007a). Data taken from table 1C, pp. 280–285, 289–294 of the study report.
* $P \leq 0.05$; ** $P \leq 0.01$; **bolded** values are considered treatment related

Table 24. Selected organ weights of F_0 and F_1 female rats in a two-generation study with saflufenacil

Organ	Mean weights ± standard deviation			
	Dose (mg/kg bw per day)			
	0	5	15	50
F_0 (*n* = 25 per group)				
Body weight (g)	*226.8 ± 10.7*	*223.3 ± 15.9*	*229.6 ± 13.6*	*228.7 ± 14.6*
Adrenal				
- absolute (mg)	72.6 ± 8.66	71.7 ± 8.72	72.0 ± 7.27	70.9 ± 7.52
- relative (/bw)	0.032 ± 0.004	0.032 ± 0.004	0.031 ± 0.003	0.031 ± 0.003
Spleen				
- absolute (g)	39.5 ± 1.49	38.9 ± 2.20	38.6 ± 1.35	38.3 ± 1.89
- relative (/bw)	0.22 ± 0.041	0.238 ± 0.048	0.232 ± 0.037	0.203 ± 0.027
Uterus				
- absolute (g)	0.798 ± 0.242	0.689 ± 0.178	0.742 ± 0.181	0.652 ± 0.318*
- relative (/bw)	0.353 ± 0.11	0.309 ± 0.081	0.324 ± 0.081	0.286 ± 0.142**
F_1 (*n* = 25 per group)				
Body weight (g)	*220.3 ± 16.8*	*218.0 ± 12.7*	*221.0 ± 18.9*	*213.0 ± 13.1*
Brain				
- absolute (g)	1.889 ± 0.098	1.87 ± 0.061	1.888 ± 0.09	1.866 ± 0.068
- relative (/bw)	0.861 ± 0.059	0.86 ± 0.053	0.858 ± 0.058	0.878 ± 0.041
Adrenal				
- absolute (mg)	75.9 ± 8.38	74.3 ± 8.35	72.0 ± 7.62	69.0 ± 9.72**
- relative (/bw)	0.035 ± 0.004	0.034 ± 0.004	0.033 ± 0.004	0.032 ± 0.004
Spleen				
- absolute (g)	0.481 ± 0.05	0.505 ± 0.059	0.499 ± 0.051	0.479 ± 0.816
- relative (/bw)	0.219 ± 0.024	0.232 ± 0.029	0.222 ± 0.025	0.225 ± 0.03
Liver				
- absolute (g)	6.679 ± 0.774	6.6 ± 0.638	6.918 ± 0.873	6.257 ± 0.816*
- relative (/bw)	3.032 ± 0.26	3.03 ± 0.27	3.129 ± 0.279	2.939 ± 0.343
Kidneys				
- absolute (g)	1.705 ± 0.127	1.691 ± 0.126	1.732 ± 0.16	1.652 ± 0.103
- relative (/bw)	0.775 ± 0.044	0.776 ± 0.049	0.785 ± 0.056	0.777 ± 0.043
Pituitary gland				
- absolute (mg)	12.12 ± 2.44	11.8 ± 1.94	11.64 ± 1.32	10.48 ± 2.29**
- relative (/bw)	0.006 ± 0.001	0.005 ± 0.001	0.005 ± 0.001	0.005 ± 0.001
Uterus				
- absolute (g)	0.607 ± 0.236	0.696 ± 0.276	0.61 ± 0.187	0.662 ± 0.248
- relative (/bw)	0.276 ± 0.106	0.32 ± 0.127	0.276 ± 0.079	0.311 ± 0.116
Ovaries				
- absolute (mg)	93.9 ± 14.33	101.3 ± 10.7	96.4 ± 14.8	99.9 ± 13.3
- relative (/bw)	0.043 ± 0.006	0.047 ± 0.005*	0.044 ± 0.006	0.047 ± 0.006*

From Schneider et al. (2007a). Data taken from table 1C, pp. 280–285, 289–294 of the study report.
* $P \leq 0.05$; ** $P \leq 0.01$

In F_1 pups, several litter and pup parameters at 50 mg/kg bw per day were adversely (statistically significant) affected by dietary exposure to saflufenacil (Table 25). The effects included a decrease in the number of liveborn pups, a higher number of stillborn pups, a reduced viability index and a lower lactation index when compared with the concurrent control values. The marginally higher number of dead pups and lower viability index in the low-dose group were regarded as spontaneous in nature and not related to treatment. There were no treatment-related effects on clinical signs or on sex ratios of live F_1 pups at birth or on lactation day 21. In F_2 pups, the number of dead pups was statistically significantly increased at 50 mg/kg bw per day. Consequently, the viability index was statistically significantly reduced in the high-dose group (87% versus 99–100% for other groups). The increased pup mortality in the high-dose group during this early stage of pre-weaning development was considered to be treatment related.

Mean body weights of F_1 male and female pups at 50 mg/kg bw per day were statistically significantly lower than those of controls during lactation days 1–14 (approximately 16% of control value), but the pups gained weight steadily (Table 26). Mean body weights of high-dose F_1 pups were about 5% less than those of the concurrent controls on lactation day 21; however, the decrease was not statistically significant. Mean body weights of F_2 male and female pups at 50 mg/kg bw per day were statistically significantly lower than those of controls during the entire lactation period. Starting with a body weight of approximately 20% less than that of the concurrent control on day 1 postpartum, the high-dose pups weighed about 9% less than the concurrent controls on lactation day 21. Body weight gain was statistically significantly decreased in high-dose pups on lactation days 1–7 (approximately 22%). Thereafter, body weight gain of these pups was essentially similar to that of the controls, although, throughout lactation, high-dose male pups on average still gained 7% less weight than control pups. No treatment-related effects on F_1 and F_2 pup body weights were noted at 5 or 15 mg/kg bw per day.

Each F_1 female pup that was selected to become the F_1 parent was evaluated for commencement of sexual maturity based on the observation of vaginal opening. The first and the last days when vaginal opening occurred were day 28 and day 36 postpartum, respectively. The mean numbers of days to reach the criterion were 31.3 ± 1.17, 31.3 ± 1.18, 32.5 ± 1.64 (statistically significant: $P \leq 0.05$) and 31.4 ± 1.96 at 0, 5, 15 and 50 mg/kg bw per day, respectively. Thus, based on the lack of a dose–response relationship, dietary exposure to saflufenacil had no adverse effects on female pup maturation. The values were also within the historical control range. Each F_1 male pup that was selected to become the F_1 parent was evaluated for commencement of sexual maturity based on the observation of preputial separation. The first and the last days when preputial separation occurred were day 40 and day 46 post-partum, respectively. The mean numbers of days to reach the criterion were 42.4 ± 1.64, 42.0 ± 1.41, 42.1 ± 1.44 and 41.7 ± 0.95 at 0, 5, 15 and 50 mg/kg bw per day, respectively. Thus, dietary exposure to saflufenacil had no adverse effects on male pup maturation. F_1 pups, on lactation day 4 but not on day 21, exhibited a dose-dependent reduction in mean platelet counts (statistically significant only in female pups at 50 mg/kg bw per day). The day 21 male pups at 50 mg/kg bw per day had decreased MCV values and increased MCHC values; both effects were marginal. The increased MCHC values were inconsistent with the kind of anaemia that was expected (i.e. hypochromic anaemia) and therefore were regarded as incidental. The decreased MCV values as the solely changed parameter of red blood cells could not be regarded as an indicator of an anaemic situation and therefore was not considered toxicologically relevant. Compared with the control, the F_2 female pups at 50 mg/kg bw per day had statistically significantly lower haemoglobin and haematocrit values. As the red blood cells were the targets of saflufenacil, the lower haemoglobin and haematocrit values in high-dose female pups were considered to be treatment related and adverse. Decreases in platelet counts were observed on lactation day 4 in male and female F_2 pups at 50 mg/kg bw per day. When assessed on lactation day 21, there were no treatment-related effects on haematology parameters. There were no treatment-related effects on serum enzyme activities in male and female F_1 and F_2 pups. The statistically significantly decreased absolute brain, thymus and/or spleen weights and relative thymus weights of the high-dose F_1 and/or F_2

Table 25. Summary of litter/pup data in a two-generation study in rats with saflufenacil

	Mean values ± standard deviation							
	F_1				F_2			
	Dose (mg/kg bw per day)							
	0	5	15	50	0	5	15	50
No. of litters with live pups	25	24	25	24	24	24	25	24
No. of pups delivered	285	273	291	**259**	247	254	279	**227**
No. of liveborn pups	283	272	288	**240****	243	254	278	**211****
No. of stillborn pups	2	1	3	**19****	4	0	1	**16****
No. of pups that died	0	5*	0	**9****	1	1	1	**12****
Sex ratio (% males)								
- day 0	51.2	46.7	46.9	53.3	46.9	46.9	53.2	49.3
- day 21	51.3	49.2	47.7	52.3	46.5	50.3	52.5	48.0
No. of pups died, days 0–4 (%)	0 (0)	5* (2)	1 (0)	**23** (10)**	0 (0)	2 (1)	1 (0)	**27** (13)**
No. of pups died, days 4–21 (%)	1 (0)	1 (1)	1 (1)	**5* (3)**	0 (0)	0 (0)	0 (0)	1 (1)
No. of pups surviving								
- days 0–4 (viability index, %)	283 (100)	267 (98)	287 (100)	**217** (90)**	242 (100)	252 (99)	277 (100)	**183** (87)**
- days 4–21 (lactation index, %)	199 (100)	189 (99)	197 (99)	**172*** (97)	187 (100)	187 (100)	200 (100)	152 (99)
Mean litter size								
- day 1	11.3 ± 1.97	11.2 ± 2.06	11.5 ± 2.04	**9.3 ± 2.82**	10.1 ± 2.23	10.5 ± 2.17	11.1 ± 1.73	**7.7 ± 3.71**
- day 4 (pre-cull)	11.3 ± 1.97	11.1 ± 2.09	11.5 ± 2.04	**9.0 ± 2.97**	10.1 ± 2.24	10.5 ± 2.21	11.1 ± 1.73	**7.6 ± 3.77**
- day 4 (post-cull)	8.0 ± 0.20	7.9 ± 0.41	7.9 ± 0.40	7.4 ± 1.71	7.8 ± 0.72	7.8 ± 1.02	8.0 ± 0	6.4 ± 2.76
- day 7	8.0 ± 0.20	7.9 ± 0.41	7.9 ± 0.40	7.3 ± 1.71	7.8 ± 0.72	7.8 ± 1.02	8.0 ± 0	6.3 ± 2.82
- day 14	8.0 ± 0.20	7.9 ± 0.41	7.9 ± 0.44	7.2 ± 1.74	7.8 ± 0.72	7.8 ± 1.02	8.0 ± 0	6.3 ± 2.82
- day 21	8.0 ± 0.20	7.9 ± 0.45	7.9 ± 0.44	7.2 ± 1.74	7.8 ± 0.72	7.8 ± 1.02	8.0 ± 0	6.3 ± 2.82

From Schneider et al. (2007a). Data taken from table 1A, pp. 161–163, 208–209 of the study report.
* $P \leq 0.05$; ** $P \leq 0.01$; **bolded** values are considered treatment related

Table 26. Summary of litter/pup body weight data in a two-generation study in rats with saflufenacil

	Mean weights ± standard deviation							
	F_1				F_2			
	Dose (mg/kg bw per day)							
	0	5	15	50	0	5	15	50
No. of litters	*25*	*24*	*25*	*23*	*24*	*24*	*25*	*22*
Pup weight (g)								
Day 1								
- Males	6.4 ± 0.61	6.3 ± 0.58	6.2 ± 0.51	**5.4 ± 0.78****	6.6 ± 0.63	6.5 ± 0.50	6.3 ± 0.62	**5.2 ± 0.82****
- Females	6.1 ± 0.56	6.0 ± 0.50	6.0 ± 0.61	**5.0 ± 0.65****	6.3 ± 0.61	6.3 ± 0.47	6.0 ± 0.63	**4.9 ± 0.72****
Day 4 (pre-cull)								
- Males	9.7 ± 1.10	9.7 ± 1.12	9.6 ± 0.92	**8.3 ± 1.44****	10.3 ± 1.08	10.1 ± 0.93	9.8 ± 1.02	**8.1 ± 1.61****
- Females	9.3 ± 0.97	9.3 ± 1.08	9.4 ± 1.05	**7.9 ± 1.19****	9.8 ± 1.03	9.8 ± 0.89	9.4 ± 0.95	**7.8 ± 1.47****
Day 4 (post-cull)								
- Males	9.7 ± 1.10	9.8 ± 1.09	9.6 ± 0.90	**8.3 ± 1.45****	10.3 ± 1.09	10.1 ± 0.95	9.8 ± 1.03	**8.1 ± 1.61****
- Females	9.3 ± 0.93	9.3 ± 1.03	9.5 ± 0.98	**8.0 ± 1.22****	9.8 ± 1.05	9.9 ± 0.88	9.5 ± 0.94	**7.8 ± 1.51****
Day 7								
- Males	15.7 ± 1.30	15.7 ± 1.58	15.8 ± 1.25	**13.4 ± 2.09****	16.3 ± 1.32	16.1 ± 1.16	15.6 ± 1.48	**13.1 ± 2.33****
- Females	15.0 ± 1.03	15.2 ± 1.47	15.5 ± 1.28	**13.0 ± 1.87****	15.6 ± 1.33	15.7 ± 1.13	15.2 ± 1.26	**12.6 ± 2.14****
Day 14								
- Males	31.3 ± 2.25	31.6 ± 2.80	31.7 ± 2.43	**29.2 ± 2.73***	32.2 ± 2.53	32.0 ± 1.65	31.3 ± 2.76	**28.3 ± 4.14***
- Females	30.4 ± 1.78	30.5 ± 2.75	31.4 ± 2.47	**28.2 ± 2.60****	31.1 ± 2.48	31.6 ± 1.76	30.4 ± 2.42	**27.5 ± 3.86****
Day 21								
- Males	49.2 ± 2.87	49.2 ± 3.64	49.8 ± 3.51	47.0 ± 4.50	50.4 ± 3.72	50.2 ± 2.48	49.4 ± 4.14	**45.4 ± 5.90****
- Females	47.7 ± 2.37	47.4 ± 3.26	48.5 ± 3.43	45.5 ± 3.69	48.2 ± 3.88	49.0 ± 2.75	47.9 ± 3.66	**43.8 ± 5.38****
Pup weight change (g)								
Days 1–4								
- Males	3.3 ± 0.59	3.4 ± 0.63	3.4 ± 0.47	2.9 ± 0.75	3.7 ± 0.54	3.5 ± 0.56	3.5 ± 0.50	**2.8 ± 0.99****

Table 26 (continued)

	Mean weights ± standard deviation							
	F_1				F_2			
	Dose (mg/kg bw per day)							
	0	5	15	50	0	5	15	50
- Females	3.2 ± 0.52	3.3 ± 0.66	3.4 ± 0.49	2.8 ± 0.66	3.5 ± 0.54	3.6 ± 0.49	3.5 ± 0.43	**2.8 ± 0.83****
Days 4–7								
- Males	6.0 ± 0.53	6.0 ± 0.67	6.1 ± 0.77	**5.1 ± 0.85****	6.0 ± 0.69	5.9 ± 0.57	5.8 ± 0.74	**4.9 ± 0.84****
- Females	5.7 ± 0.51	5.9 ± 0.65	6.0 ± 0.73	**5.0 ± 0.87****	5.8 ± 0.70	5.9 ± 0.57	5.7 ± 0.63	**4.8 ± 1.08****
Days 7–14								
- Males	15.6 ± 1.47	15.8 ± 1.52	16.0 ± 1.52	15.8 ± 2.00	16.0 ± 1.68	16.0 ± 1.09	15.6 ± 1.56	15.2 ± 2.24
- Females	15.4 ± 1.31	15.2 ± 1.56	15.8 ± 1.59	15.2 ± 1.30	15.5 ± 1.42	15.8 ± 1.24	15.2 ± 1.49	14.9 ± 1.78
Days 14–21								
- Males	17.9 ± 1.74	17.7 ± 1.51	18.1 ± 1.51	17.8 ± 2.05	18.1 ± 1.75	18.1 ± 1.21	18.1 ± 1.69	17.1 ± 2.07
- Females	17.3 ± 1.38	16.9 ± 1.45	17.1 ± 1.39	17.3 ± 1.49	17.2 ± 1.90	17.4 ± 1.46	17.5 ± 1.60	16.4 ± 1.89
Days 4–21								
- Males	39.5 ± 2.54	39.6 ± 2.79	40.2 ± 2.98	38.7 ± 3.65	40.1 ± 3.19	40.1 ± 2.24	39.6 ± 3.61	**37.3 ± 4.56***
- Females	38.4 ± 1.99	38.0 ± 2.49	39.1 ± 2.71	37.6 ± 2.88	38.4 ± 3.34	39.2 ± 2.44	38.5 ± 3.25	36.1 ± 4.21

From Schneider et al. (2007a). Data taken from table 1A, pp. 164–167, 210–213 of the study report.
* $P \leq 0.05$; ** $P \leq 0.01$; **bolded** values are considered treatment related

pups were considered secondary to the lower pup body weights in this group. They were not considered to be adverse or toxicologically relevant. A number of F_1 pups showed findings at gross necropsy, such as postmortem autolysis, sloped incisors, anasarca, small thymus, abnormal lung lobulation, infarct of liver, yellowish discoloured liver, empty stomach, hydronephrosis, infarct of kidney, dilated renal pelvis, distended urinary bladder, dilated ureter, small testis, anorchia and haemorrhagic testis. However, these findings were not considered treatment related, as findings were observed only in single animals and/or can be found in the historical control data at comparable or even higher incidences. Discoloured liver was observed in three animals from two litters in the F_1 generation and five animals from four litters in the F_2 generation. A number of F_2 pups showed findings at gross necropsy, such as postmortem autolysis, haemorrhagic thymus, diaphragmatic hernia, misshapen spleen, infarct of liver, dilated renal pelvis, small testis, malpositioned testis and haemorrhagic epididymis. All of these pup necropsy findings occurred without any relation to dosing, and most can be found in the historical control data at comparable or even higher incidences. The number of affected pups per litter showing yellowish discoloured livers or intestines was increased at 50 mg/kg bw per day (significant increase in intestines only). Discoloured liver was observed in three pups from two litters in the F_1 generation and five pups from four litters in the F_2 generation. Based on the mode of action of saflufenacil for which the liver is a target of toxicity due to porphyrin accumulation, the significance of this finding is unclear in the absence of other evidence of hepatotoxicity. There were no treatment-related microscopic findings noted in any of the dose groups of any generation.

The NOAEL for parental systemic toxicity was 15 mg/kg bw per day, based on adverse effects on feed intake, body weight gain and MHA seen at 50 mg/kg bw per day. The NOAEL for reproductive toxicity was 50 mg/kg bw per day, the highest dose tested. The NOAEL for offspring toxicity was 15 mg/kg bw per day, based on the increased number of stillborn pups, increased pup mortality during the early phase of lactation, reduced pup weight gains and indications of anaemia seen at 50 mg/kg bw per day. The study authors (Schneider et al., 2007b) concluded that the NOAEL for parental systemic toxicity was 5 mg/kg bw per day. However, marginally decreased haemoglobin (F_0 and F_1 males and females), haematocrit and MCV (F_0 and F_1 males) and decreased MCH, protein and albumin (F_1 males) were seen at 15 mg/kg bw per day. These effects were not considered adverse, as the magnitude of the decreases was small and there were no other adverse effects observed in the F_0 and F_1 parental animals.

(b) Developmental toxicity

Rats

In a developmental toxicity study, saflufenacil (purity 93.8%) was administered to 25 female Wistar rats per dose by gavage at a dose level of 0, 5, 20 or 60 mg/kg bw per day from days 6 through 19 of gestation. The test substance suspension for gavage administration was prepared in 1.0% carboxymethylcellulose in doubly distilled water. The dosing solution was analysed for stability, homogeneity and concentration. The animals were checked for mortality or clinical signs at least daily. Body weight data were recorded on gestation days 0, 1, 3, 6, 8, 10, 13, 15, 17, 19 and 20. With the exception of day 0, feed consumption was determined on the same days as body weight. Dams were sacrificed on day 20 of gestation. Blood was taken from all females prior to sacrifice for haematological and clinical chemistry measurements. Gross pathology was conducted on all females. Livers and spleens were weighed and prepared for histopathological examination. At necropsy, each fetus was weighed, sexed and examined macroscopically for any external findings. The viability of the fetuses and the condition of the placenta, umbilical cords, fetal membranes and fluids were examined. Individual placental weights were recorded. Thereafter, the fetuses were sacrificed by subcutaneous injection of a pentobarbital solution. After these examinations, approximately half of the fetuses per dam were eviscerated, skinned and placed in ethyl alcohol, and the remaining fetuses were placed in Harrison's fluid for fixation and further evaluation.

The dosing solution was stable for 96 hours at room temperature. The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable (90.7–103.2% of the nominal concentrations). There were no treatment-related effects on body weight, body weight gain, feed consumption or gross necropsy. At the end of the administration period, significantly decreased values for haemoglobin (94% of control), haematocrit (95% of control), MCV (95% of control) and MCH (95% of control) were found in the peripheral blood of the high-dose dams (Table 27). Statistically significantly decreased haematocrit (95% of control) and decreased haemoglobin concentration (96% of control; not statistically significant but dose related) were also observed in the mid-dose group (20 mg/kg bw per day). The changes in haematological parameters seen at 20 and 60 mg/kg bw per day are small in magnitude but considered to be adverse effects because of the primary mode of action of saflufenacil. No treatment-related changes were seen in the other red blood cell parameters of treated animals. Haematology examinations also revealed slightly, but statistically significantly ($P < 0.05$), increased white blood cell counts (117% of control) in the circulation of the high-dose dams. Because of the low magnitude of change, this finding was considered not to be of toxicological or biological relevance. There were no treatment-related changes in clinical chemistry parameters. There was an increase in liver porphyrins at and above 5 mg/kg bw per day, but the adverse nature of the change in porphyrins is unknown in the absence of other liver changes. There were no treatment-related changes at gross necropsy and no organ weight changes in the liver or spleen.

There was a decrease in fetal body weights at 20 mg/kg bw per day, as summarized in Table 28. Although there was an increase in live births per dam at the same dose, it does not entirely account for the reduced fetal weights and is considered treatment related and adverse.

Only one external malformation (cleft palate) was seen in one fetus of the 20 mg/kg bw per day test group, which was not considered to be treatment related. There was no increase in visceral malformations or variations. There was a treatment-related increase in the number of skeletal malformations (anomalies) at 60 mg/kg bw per day, with 10 individuals and seven litters affected (Table 29). The malformations consisted of bent scapulae, radii, ulnas and femurs, malpositioned and bipartite sternebrae and thick humeri. At 20 mg/kg bw per day, there was a single incidence of bent scapula. As this occurred in the absence of historical control findings, the malformation was considered treatment related. However, the incidence of bent scapula was considered delayed ossification and therefore unlikely to occur following a single dose. The litters of every dose group, including control, had skeletal variations. However, the proportions of individuals affected were higher in the 60 mg/kg bw per day dose groups, with all individuals affected, a finding above that of historical controls. At doses of 20 mg/kg bw per day and above, there were increases in incomplete ossification of the skull, nasal area and thoracic centrum and wavy ribs. At 60 mg/kg bw per day, there were increases in supraoccipital holes, bassioccipital holes, incomplete ossification of the hyoid and thoracic centrum, dumbbell ossification of the lumbar centrum and bipartite ossification of the sternebrae.

The maternal toxicity LOAEL is 60 mg/kg bw per day, based on statistically significantly decreased haematocrit (95% of control). The maternal toxicity NOAEL is 20 mg/kg bw per day. The developmental toxicity LOAEL is 20 mg/kg bw per day, based on decreased fetal body weights in males and females and increased skeletal anomalies (only one finding of bent scapula; unlikely due to single dose) and variations. The developmental toxicity NOAEL is 5 mg/kg bw per day (Schneider et al., 2007b; Schneider, 2008).

Rabbit

In a developmental toxicity study, saflufenacil (purity 93.8%) was administered to 25 female Himalayan rabbits per dose by gavage at a dose level of 0, 50, 200 or 600 mg/kg bw per day from days 6 through 28 of gestation. The test substance was given via gavage as an aqueous suspension in 1% carboxymethylcellulose. The dosing solution was analysed for stability, homogeneity and concentration.

Table 27. Select haematological parameters in the dams

	Mean values ± standard deviation			
	Dose (mg/kg bw per day)			
	0	5	20	60
Number of dams	*24*	*23*	*22*	*24*
White blood cells (10^9/l)	4.38 ± 1.21	4.57 ± 0.96 (↑4.33)	4.50 ± 0.96 (↑2.74)	5.14 ± 1.06* (↑17.35)
Haemoglobin (mmol/l)	6.9 ± 0.7	6.9 ± 0.5 (0.0)	6.6 ± 0.3 (↓4.35)	**6.5 ± 0.6* (↓5.80)**
Haematocrit (l/l)	0.320 ± 0.030	0.321 ± 0.024 (↑0.31)	0.303 ± 0.015* (↓5.31)	**0.304 ± 0.027* (↓5.00)**
MCV (fl)	55.0 ± 1.5	54.6 ± 1.5 (↓0.73)	54.6 ± 1.4 (↓0.73)	**52.5 ± 1.5** (↓4.55)**
MCH (fmol)	1.19 ± 0.04	1.17 ± 0.04 (↓1.68)	1.19 ± 0.04 (0.0)	**1.13 ± 0.04** (↓5.04)**
Platelets (10^9/l)	983 ± 172	984 ± 132 (↑0.10)	960 ± 143 (↓2.34)	945 ± 161 (↓3.87)

From Schneider et al. (2007b). Data extracted from pp. 82–83 of the study report.
MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume; * $P < 0.05$; ** $P < 0.01$; **bolded** values are considered to be treatment related

Table 28. Caesarean section observations in rat developmental toxicity study with saflufenacil

Observation	Dose (mg/kg bw per day)			
	0	5	20	60
No. of animals assigned (mated)	25	25	25	25
No. of animals pregnant	24	23	22	24
- pregnancy rate (%)	96	92	88	96
No. non-pregnant	1	2	3	1
Maternal wastage				
- no. died	0	0	0	0
- no. died pregnant	0	0	0	0
- no. died non-pregnant	0	0	0	0
- no. aborted	0	0	0	0
- no. premature delivery	0	0	0	0
Total no. of corpora lutea	231	218	221	240
- no. of corpora lutea/dam	9.6 ± 176	9.5 ± 195	10.0 ± 1.46	10.0 ± 1.640
Total no. of implantations	185	191	207	203
- no. of implantations/dam	7.7 ± 3.26	8.3 ± 3.07	9.4 ± 1.71	8.5 ± 2.69
Total no. of litters	22	23	22	22
Total no. of live fetuses	174	179	196	187
- no. of live fetuses/dam	7.9 ± 2.67	7.8 ± 2.91	8.9 ± 1.97	8.5 ± 1.74
Total no. of dead fetuses	0	0	0	0
- no. of dead fetuses/dam	0	0	0	0
Total no. of resorptions	11	12	11	16
- early	11	11	11	14
- late	0	1	0	2
No. of resorptions/dam	0.5 ± 0.66	0.5 ± 0.59	0.5 ± 1.22	0.7 ± 1.05
- early	0.5 ± 0.66	0.5 ± 0.51	0.5 ± 1.22	0.6 ± 1.06
- late	0.0 ± 0.00	0.0 ± 0.21	0.0 ± 0.00	0.1 ± 0.28
No. of litters with total resorptions	2	0	0	2
Mean fetal weight (g)	3.7 ± 0.44	3.7 ± 0.21 (0)	**3.4 ± 0.32* (↓8.1)**	**3.1 ± 0.25** (↓16.2)**
- males	3.8 ± 0.49	3.8 ± 0.19 (0)	**3.5 ± 0.31 (↓7.9)**	**3.2 ± 0.30** (↓15.8)**
- females	3.6 ± 0.44	3.7 ± 0.21 (↑2.8)	**3.4 ± 0.34* (↓5.6)**	**3.0 ± 0.30** (↓16.7)**
Sex ratio (% male)	52.9	49.2	47.4	54.5
Pre-implantation loss (%)	19.6 ± 29.93	16.0 ± 24.11	6.1 ± 11.51	14.1 ± 26.34
Post-implantation loss (%)	14.0 ± 28.86	5.7 ± 6.40	5.1 ± 12.30	14.8 ± 28.73

From Schneider et al. (2007b). Data obtained from pp. 79–81 of the study report.
* $P < 0.05$; ** $P < 0.01$; **bolded** values are considered to be treatment related

Table 29. Skeletal examinations in rat developmental toxicity study with saflufenacil

Observations[a]	Dose (mg/kg bw per day)			
	0	5	20	60
No. of fetuses (litters) examined	94 (22)[b]	97 (23)	106 (22)	98 (22)
Malformations				
No. of fetuses (litters) affected	1 (1)	0 (0)	4 (4) [3.8 (18.2)]	10 (7)* [10.2 (31.8)]
HC: Total skeletal malformations	0.0–5.1 (0.0–25.0)			
Bent scapula: Cartilage present	0 (0)	0 (0)	1 (1)	5 (3)*
HC: Bent scapula	No historical findings			
Malpositioned and bipartite sternebra: Unchanged cartilage	0 (0)	0 (0)	1 (1) [0.9 (4.5)]	2 (2) [2.0 (9.0)]
HC: Malpositioned and bipartite sternebra: Unchanged cartilage	0.0–1.7 (0.0–8.0)			
Thick humerus: Cartilage present	0 (0)	0 (0)	0 (0)	5 (3)*
HC: Thick humerus	No historical findings			
Bent radius: Cartilage present	0 (0)	0 (0)	0 (0)	4 (2)
HC: Bent radius	No historical findings			
Bent ulna: Cartilage present	0 (0)	0 (0)	0 (0)	3 (2)
HC: Bent ulna	No historical findings			
Bent femur: Cartilage present	0 (0)	0 (0)	0 (0)	3 (2)
HC: Bent femur	No historical findings			
Misshapen lumbar vertebrae	0 (0)	0 (0)	1 (1)	0 (0)
Notched scapula: Cartilage present	0 (0)	0 (0)	1 (1)	0 (0)
Cleft sternum: Split cartilage	0 (0)	0 (0)	1 (1)	0 (0)
Variations				
No. of fetuses (litters) affected	90 (22)	94 (23)	105 (22) [99.0 (100.0)]	98 (22)* [100.0 (100.0)]
HC: Total skeletal variations	88.0–99.2 (100.0–100.0)			
Supraoccipital hole(s)	26 (13)	30 (15)	33 (18) [31.4 (81.8)]	49 (20)** [50.0 (90.9)]
HC: Supraoccipital hole(s)	8.0–59.3 (33.3–100.0)			
Incomplete ossification of skull: Unchanged cartilage	3 (3)	5 (2)	5 (4) [4.8 (18.2)]	7 (5) [7.1 (22.7)]
HC: Incomplete ossification of skull	0–10.8 (0.0–30.4)			
Bassioccipital hole(s)	1 (1)	0 (0)	1 (1)	5 (4) [5.1 (18.2)]
HC: Bassioccipital hole(s)	0–4.0 (0.0–12.5)			
Incomplete ossification of hyoid: Cartilage present	0 (0)	0 (0)	0 (0)	2 (2) [2.0 (9.1)]
HC: Incomplete ossification of hyoid	0–3.6 (0–8.3)			
Incomplete ossification of nasal bone: Unchanged cartilage	0 (0)	0 (0)	4 (4)*	15 (9)**
HC: Incomplete ossification of nasal bone	No historical findings			
Incomplete ossification of thoracic centrum: Unchanged cartilage	2 (2)	1 (1)	5 (3) [4.7 (13.6)]	12 (9)** [12.2 (40.9)]

Table 29 (continued)

Observations[a]	Dose (mg/kg bw per day)			
	0	5	20	60
HC: Incomplete ossification of the thoracic centrum	0.0–1.0 (0.0–4.6)			
Dumbbell ossification of lumbar centrum: Unchanged cartilage	3 (3)	4 (4)	1 (1)	6 (6) [6.1 (27.3)]
HC: Dumbbell ossification of lumbar centrum	0.0–2.0 (0.0–8.7)			
Incomplete ossification of sternebra: Unchanged cartilage	56 (21)	64 (21)	73 (20) [68.9 (90.9)]	76 (21)* [77.6 (95.5)]
HC: Incomplete ossification of sternebra	38.5–74.5 (70–100.0)			
Misshapen sternebra: Unchanged cartilage	39 (20)	37 (20)	40 (19)	64 (20)* [65.3 (90.9)]
HC: Misshapen sternebra	7.7–50.0 (37.5–96)			
Bipartite ossification of sternebra: Unchanged cartilage	0 (0)	0 (0)	0 (0)	2 (2) [2.0 (9.1)]
HC: Bipartite ossification of sternebra	0–4.9 (0.0–13.0)			
Wavy rib	17 (9)	11 (7)	34 (16)* [33.3 (72.7)]	51 (17)** [52.0 (77.3)]
HC: Wavy rib	1.0–9.3 (4.8–26.1)			
Incomplete ossification of pubis: Cartilage present	0 (0)	0 (0)	1 (1)	2 (1)
Incomplete ossification of ischium: Cartilage present	0 (0)	0 (0)	1 (1)	1 (1)

From Schneider et al. (2007 b). Data extracted from pp. 100–123 of the study report; historical control (HC) data from pp. 372–377 of the study report.

* $P < 0.05$; ** $P < 0.01$; **bolded** values are considered to be treatment related

[a] Some observations may be grouped together. [ ] percentage of fetuses and litters with finding in that treatment group.

[b] Fetal (litter) incidence.

The animals were checked for mortality or clinical signs at least daily. Body weight data were recorded on days 0, 2, 4, 6, 9, 11, 14, 16, 19, 21, 23, 25, 28 and 29 post-insemination. The consumption of feed was determined daily during days 1–29 post-insemination. Dams were sacrificed on day 29 post-insemination. Blood was taken from all females prior to sacrifice for haematological and clinical chemistry measurements. Gross pathology was conducted on all females. Liver samples were taken from five pregnant rabbits and their fetuses to determine liver porphyrins. Livers and spleens were weighed and prepared for histopathological examination. At necropsy, each fetus was weighed, sexed and examined macroscopically for any external findings. The viability of the fetuses and the condition of the placenta, umbilical cords, fetal membranes and fluids were examined. Individual placental weights were recorded. After the fetuses had been sacrificed, the abdomen and thorax were opened in order to be able to examine the organs in situ before they were removed. The heart and the kidneys were sectioned in order to assess the internal structure. The sex of the fetuses was determined by internal examination of the gonads. The heads of approximately one half of the fetuses per dam were fixed in Bouin's solution and processed and assessed according to Wilson's method. After skinning, all fetuses (including those without heads) were fixed in ethyl alcohol for skeletal examinations.

The dosing preparation was stable for 96 hours at ambient temperature. The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable (greater than 90% and less than 110% of the nominal concentrations). Clinical signs were limited to the 600 mg/kg bw per day dose group, with lateral positioning, poor

general state, abortion, blood in bedding, discoloured and no urination and reduced or no defecation. Two high-dose dams were sacrificed in moribund condition on days 17 and 24, respectively. One dam from the 200 mg/kg bw per day dose group was found dead on gestation day 23, and one dam from the 600 mg/kg bw per day dose group was found dead on gestation day 27. One 50 mg/kg bw per day dam was sacrificed following abortion on gestation day 23. Two 600 mg/kg bw per day dams were sacrificed following abortion on gestation day 24, another on gestation day 25 and a final dam following abortion on gestation day 29 just prior to scheduled sacrifice. The increased mortality rate at 600 mg/kg bw per day, with four abortions, two moribund sacrifices and one premature death, is considered to be treatment related. There were no statistically significant or biologically relevant differences between the controls and the substance-treated dams in terms of body weights and body weight gain during the study. Feed consumption was decreased in the 600 mg/kg bw per day dose group from gestation day 6 to gestation day 18. Overall feed consumption was decreased in the 600 mg/kg bw per day dose group. There were no treatment-related, adverse organ weight changes. At doses at or above 200 mg/kg bw per day, there were stomach ulcerations, lack of faeces and assorted findings on implantations in dams sacrificed moribund. At 600 mg/kg bw per day, there was an increase in pale livers and kidneys, empty stomachs, enlarged bladders and assorted findings on implantations in dams that aborted. There were no treatment-related changes in the haematological parameters measured. Only slight but statistically significantly increased white blood cells and decreased platelets (high-dose does only) were observed. The haemoglobin, haematocrit, MCV, MCH and MCHC measures were unaffected by treatment at any dose level. There were no treatment-related changes in the clinical chemistry parameters. On day 29 post-insemination, total porphyrin concentrations in the liver of treated dams were statistically significantly increased compared with the animals of the control group. The liver porphyrin changes were not considered to be treatment related or adverse, as there were no other clinical, haematology or clinical chemistry changes at doses of or below 200 mg/kg bw per day. There was a treatment-related increase in maternal wastage at 600 mg/kg bw per day, with increases in dams that were found dead or sacrificed moribund and an increase in abortions. At 600 mg/kg bw per day, there was also a decrease in total litters, total live fetuses and live fetuses per dam. There were no effects on resorptions, fetal body weights, sex ratios or post-implantation loss. There were decreases in the total number of corpora lutea and corpora lutea per dam, total implantations and implantations per dam at 600 mg/kg bw per day, but as treatment did not commence until after implantation, the change is not treatment related. The findings are summarized in Table 30.

Liver porphyrins were increased at doses of 200 mg/kg bw per day and above in male and female fetuses. The nature of the changes in the total liver porphyrins was unclear due to a lack of haematological parameters measured in fetuses in the study. There were no treatment-related changes observed in the external examinations. There were no treatment-related changes to visceral malformations. There was a slight increase in the number of litters with skeletal malformations at doses at and above 200 mg/kg bw per day, especially when considering the reduced litters at 600 mg/kg bw per day. At 200 mg/kg bw per day, there was one fetus with sternebrae fused into a bony plate and one fetus with broken sternebra, ribs, humerus, tibia and fibula and knobby ulna and rib. At 600 mg/kg bw per day, there was one fetus with small interparietal and supraoccipital bones and another fetus with lumbar hemivertebra. However, as these were single incidences and there were no patterns of malformation or changes to the number of variations or unclassified changes, these malformations were not considered to be treatment related.

The maternal toxicity LOAEL was 600 mg/kg bw per day, based on increased mortality, clinical signs (lateral positioning, poor general state, abortion, blood in bedding, discoloured or no urination and reduced or no defecation) and increased necropsy findings (stomach ulcerations, lack of faeces, increase in pale livers and kidneys, empty stomachs, enlarged bladders and assorted findings on implantations in dams that aborted or were moribund). The NOAEL for maternal toxicity was 200 mg/kg bw per day. The developmental toxicity LOAEL was 600 mg/kg bw per day, based on a decrease in total litters and total live fetuses and live fetuses per dam. The developmental toxicity NOAEL was 200 mg/kg bw per day (Schneider et al., 2006).

Table 30. Caesarean section observations in a rabbit developmental toxicity study with saflufenacil

Observation	Dose (mg/kg bw per day)			
	0	50	200	600
No. of animals assigned (mated)	25	25	25	25
No. of animals pregnant	25	24	24	24
- pregnancy rate (%)	100	96	96	96
No. non-pregnant	0	1	1	1
Maternal wastage				
- no. died[a]	0	1	1	**7****
- no. died pregnant	0	1	1	**7**
- no. died non-pregnant	0	0	0	0
- no. aborted	0	1	0	**4**
- no. premature delivery	0	0	0	0
Total no. of corpora lutea	204	186 (↓8.8)	185 (↓9.3)	126 (↓38.2)
- no. of corpora lutea/dam	8.2 ± 1.60	8.1 ± 1.35 (↓1.2)	8.0 ± 1.55 (↓2.4)	7.4 ± 1.37 (↓9.8)
Total no. of implantations	169	173 (↑2.4)	158 (↓6.5)	104 (↓38.9)
- no. of implantations/dam	6.8 ± 2.44	7.5 ± 1.08 (↑10.3)	6.9 ± 2.07 (↑1.5)	6.1 ± 1.41 (↓10.3)
Total no. of litters	25	23 (↓8)	23 (↓8)	**17** (↓32)**
Total no. of live fetuses	157	162 (↑3.2)	150 (↓4.5)	**96 (↓38.9)**
- no. of live fetuses/dam	6.3 ± 2.19	7.0 ± 1.02 (↑11.1)	6.5 ± 2.04 (↑3.2)	**5.6 ± 1.11 (↓11.1)**
Total no. of dead fetuses	2	0	0	0
Total no. of resorptions	10	11	8	8
- early	7	7	6	8
- late	3	4	2	0
Resorptions/dam	0.4 ± 0.58	0.5 ± 0.67	0.3 ± 0.49	0.5 ± 0.80
- early	0.3 ± 0.46	0.3 ± 0.56	0.3 ± 0.45	0.5 ± 0.80
- late	0.1 ± 0.33	0.2 ± 0.39	0.1 ± 0.29	0.0 ± 0.00
No. of litters with total resorptions	0	0	0	0
Mean fetal weight (g)	39.6 ± 4.43	37.1 ± 2.93 (↓6.3)	38.1 ± 6.65 (↓3.8)	39.7 ± 3.09 (↑0.3)
- males	39.8 ± 4.32	37.0 ± 3.57 (↓7.0)	37.9 ± 7.01 (↓4.8)	39.5 ± 2.72 (↓0.8)
- females	39.0 ± 4.71	36.9 ± 3.19 (↓5.4)	37.9 ± 5.71 (↓2.8)	39.3 ± 3.61 (↑0.8)
Sex ratio (% male)	50.3	42.0 (↓16.5)	51.3 (↑2.0)	46.9 (↓6.8)
Pre-implantation loss (%)	17.9 ± 24.23	6.4 ± 7.85 (↓64.2)	16.0 ± 18.57 (↓10.6)	16.9 ± 14.49 (↓5.6)
Post-implantation loss (%)	5.8 ± 8.11	6.0 ± 8.26 (↑3.4)	5.0 ± 7.40 (↓13.8)	6.4 ± 9.92 (↑10.3)

From Schneider et al. (2006). Data extracted from pp. 80–82 and 92 of the study report.
** $P < 0.01$; **bolded** values are considered to be treatment related
[a] Includes animals found dead, sacrificed moribund and sacrificed following abortion.

2.6 Special studies

(a) Acute neurotoxicity

In an acute neurotoxicity study, saflufenacil (purity 93.8%) was administered to Wistar rats, 10 of each sex per group, by oral gavage at 0, 125, 500 or 2000 mg/kg bw. The test substance was suspended in drinking-water containing 0.5% carboxymethylcellulose. The treated rats were observed for 2 weeks. Neurobehavioural assessment (FOB and motor activity testing) was performed on days

−7, 0, 7 and 14. Dosing solutions were analysed for stability, homogeneity and concentration. All animals were observed for mortality and clinical signs twice daily on working days and once during the weekends and holidays. Feed consumption was determined weekly. Body weights were recorded on days −7, 0, 7 and 14. At study termination, five rats of each sex per group were euthanized and perfusion fixed for neuropathological examination.

The test substance was stable in the vehicle for 96 hours at room temperature. The test substance was homogeneous (91.1% with standard deviation of 1%). Mean concentrations were within 10% of the nominal concentrations. The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the rats was acceptable. All rats survived the duration of the study period. There were no treatment-related effects on clinical signs of toxicity, feed consumption and feed efficiency, body weights and body weight gain, or FOB. Males at 125 mg/kg bw showed reduced hindlimb grip strength on day 0. Males at 500 mg/kg bw showed increased hindlimb grip strength on day 7. Both findings were inconsistent single occurrences without any dose–response relationship and therefore were assessed as incidental and not related to treatment. The only noted finding was a moderately decreased motor activity in high-dose males. However, the decreased motor activity was statistically significant only when evaluated over the entire summary interval period and was noted at only the day 0 time point. The finding was not accompanied by any other neuropathological changes and was considered to be a reflection of a mild and transient general systemic toxicity and not a substance-specific neurotoxic effect. At terminal sacrifice, brain weight was not affected. Gross and histopathological examination of the brain or other nervous tissues revealed no treatment-related changes.

Based on the absence of adverse neurotoxic effects, the NOAEL for neurotoxicity was 2000 mg/kg bw in male and female rats. For systemic toxicity, the LOAEL was 2000 mg/kg bw for the males, based on decreased motor activity, representing mild and transient systemic toxicity. A LOAEL for the female was not observed. The systemic toxicity NOAELs for the male and female rats were 500 and 2000 mg/kg bw, respectively (Kaspers, Kaufmann & van Ravenzwaay, 2007).

(b) Short-term study of neurotoxicity

Rats

In a 90-day oral neurotoxicity study, Wistar rats (10 of each sex per group) were administered saflufenacil (purity 93.8%) daily in the diet at 0, 50, 250, 1000 (males) or 1350 (females) ppm (equal to 0, 3.3, 16.6 and 66.2 mg/kg bw per day for males and 0, 3.9, 19.4 and 101.0 mg/kg bw per day for females, respectively). Neurobehavioural assessment (FOB and motor activity testing) was performed on days −7, 1, 22, 50 and 85. At study termination, five rats of each sex per group were euthanized and perfusion fixed. The brain and other nervous tissues were processed for histopathological examination. Diets were prepared every 4 weeks. The stability, homogeneity and concentrations were measured analytically. The rats were examined for signs of toxicity and mortality twice a day on weekdays and once a day on Saturdays, Sundays and public holidays. Body weights were recorded prior to the treatment and weekly thereafter. Feed consumption was recorded weekly. Haematological parameters were evaluated at termination.

The test substance was homogeneously distributed and was stable for 49 days at room temperature. The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the rats was acceptable. There were no deaths observed in the study. Urine-smeared anogenital region was observed in one female at 1350 ppm and in one male at 1000 ppm. This finding was not considered to be toxicologically significant. The body weights of high-dose males and females were lower than those of the concurrent controls, attaining statistical significance on several time intervals. The overall body weight gains for the high-dose male and female rats were 14.0% and 15.9% lower than the control values at the end of the study period (Table 31). Feed consumption was decreased in high-dose males, occasionally attaining

Table 31. Mean body weights and body weight gains during 90 days of dietary administration of saflufenacil to rats

	Mean values ± standard deviation							
	Males (n = 10 per group)				Females (n = 10 per group)			
	Dietary concentration (ppm)							
	0	50	250	1000	0	50	250	1350
Body weight (g)								
Day 0	195.8 ± 10.0	199.1 ± 13.0	190.2 ± 11.1	196.8 ± 9.4	152.5 ± 7.0	150.4 ± 8.7	150.7 ± 9.0	149.3 ± 10.2
Day 91	404.4 ± 25.0	417.4 ± 25.3	384.3 ± 34.8	376.3 ± 35.5 (−6.9%)	250.5 ± 14.7	240.6 ± 16.2	246.9 ± 24.6	231.7 ± 17.2 (−7.8%)
Body weight change (g)								
Days 0–91	208.6 ± 23.4	218.3 ± 20.0	194.1 ± 32.0	**179.5 ± 34.2 (−14.0%)**	98.0 ± 16.4	90.3 ± 16.9	96.2 ± 19.0	**82.5 ± 17.9 (−15.9%)**

From Kaspers et al. (2007g). Data taken from table 1A, pp. 71–80 of the study report.
Bolded values are considered to be treatment related.

Table 32. Selected haematological data after 90-day dietary administration of saflufenacil to rats

	Mean values ± standard deviation							
	Males (n = 10 per group)				Females (n = 10 per group)			
	Dietary concentration (ppm)							
	0	50	250	1000	0	50	250	1350
Haemoglobin (mmol/l)	9.1 ± 0.3	9.1 ± 0.2	9.2 ± 0.4	**7.9 ± 0.4****	9.1 ± 0.3	8.8 ± 0.2	8.8 ± 0.3	**8.2 ± 0.4***
Haematocrit (%)	40.6 ± 1.2	40.6 ± 1.6	41.7 ± 1.6	**36.8 ± 1.9****	40.9 ± 1.5	40.2 ± 1.3	39.6 ± 1.1	37.8 ± 1.7
MCV (fl)	49.2 ± 1.2	51.2 ± 1.1*	47.4 ± 1.6	**44.5 ± 2.6****	53.3 ± 0.9	52.7 ± 0.9	53.3 ± 1.1	**47.4 ± 2.8***
MCH (fmol)	1.11 ± 0.04	1.15 ± 0.06	1.04 ± 0.04*	**0.96 ± 0.06****	1.18 ± 0.03	1.16 ± 0.02	1.19 ± 0.02	**1.03 ± 0.07****
MCHC (mmol/l)	22.5 ± 0.29	22.4 ± 0.77	21.9 ± 0.31	21.6 ± 0.24**	22.2 ± 0.33	22.0 ± 0.26	22.3 ± 0.19	**21.7 ± 0.54***

From Kaspers et al. (2007g). Data taken from table 1B, pp. 189–190 of the study report.
* $P \leq 0.05$; ** $P \leq 0.01$; **bolded** values are considered to be treatment related

statistical significance. No difference in feed consumption was noted between the high-dose males and controls towards the end of the study. Feed consumption was statistically significantly decreased in the high-dose females throughout the study. There were no treatment-related ophthalmoscopic findings (part of FOB measurements). Treatment-related decreases in haemoglobin, haematocrit (males only), MCV, MCH and MCHC were observed in high-dose animals at the end of the study (Table 32). These changes were regarded to be associated with moderate to severe anaemia. Other changes were not considered toxicologically significant because of the small magnitude of changes, lack of a dose–response relationship and/or inconsistency of the findings.

There were no treatment-related effects on the absolute or relative brain weights. There were no gross lesions that were attributable to the treatment. There were no treatment-related histopathological findings. A single (grade 1) "axonal degeneration" was recorded in the peripheral nerves of one high-dose female. This single finding was regarded as incidental or spontaneous in nature and not related to treatment, as single nerve fibre degenerations have been reported to occur spontaneously in central and peripheral nerve fibres in short-term neurotoxicity studies in rats. Neurotoxicity assessment, including an extensive FOB, motor activity, brain weights, as well as gross pathology and histopathology of the brain and other nervous tissues, did not reveal any adverse findings.

The NOAELs for neurotoxicity for the males and females were 1000 and 1350 ppm, respectively (equal to 66.2 and 101.0 mg/kg bw per day for males and females, respectively). A LOAEL for neurotoxicity was not established. The LOAELs for general systemic toxicity for the males and females were 1000 and 1350 ppm, respectively (equal to 66.2 and 101.0 mg/kg bw per day in males and females, respectively), based on treatment-related decreases in haemoglobin, haematocrit, MCV, MCH and MCHC. The NOAEL for systemic toxicity was 250 ppm (equal to 16.6 and 19.4 mg/kg bw per day in males and females, respectively) (Kaspers et al., 2007g).

(c) Immunotoxicity

Saflufenacil (purity 93.8%) was administered in the diet to groups of eight male C57BL/6J Rj mice at a dose level of 0, 50, 125 or 250 ppm (equal to 0, 10, 27 and 52 mg/kg bw per day, respectively) for a 4-week period. In addition, a concurrent positive control group of eight male C57BL/6J Rj mice received cyclophosphamide monohydrate in water at a dose level of 10 mg/kg bw per day by gavage for 4 weeks. All animals were immunized with a 0.5 ml intraperitoneal injection of sheep red blood cells on day 23. On day 29, blood was collected for primary T cell–dependent antibody response (anti-sheep red blood cell immunoglobulin M [IgM] enzyme-linked immunosorbent assay evaluation) and necropsied. Selected tissues (liver, spleen and thymus) were removed, weighed and prepared for histopathological examination.

No treatment-related effects on clinical signs, mortality, body weight, body weight gain or feed consumption were observed in saflufenacil treatment groups. Decreased red blood cell counts, haemoglobin and haematocrit values were observed in the 125 and 250 ppm dose groups. In mice of the positive control group, in addition to the decreased red blood cell, haemoglobin and haematocrit values, MCV and MCH were increased. ALT activity was increased in the 250 ppm dose group. Alkaline phosphatase activity was reduced in the 125 and 250 ppm dose groups and also in the positive control group. There were no treatment-related effects on the absolute or relative spleen and thymus weights in any saflufenacil-treated groups. No histopathological findings in the spleen and thymus were noted in the saflufenacil-treated mice. As expected, there were treatment-related increases in the spleen and thymus weights in the positive control group. Increased liver weight was observed at 250 ppm. Histopathological investigation of the liver revealed a slight to moderate centrilobular fatty change in almost all animals (seven out of eight) at 250 ppm. Additionally, two animals showed minimal lymphoid infiltration, and a single animal showed minimal extramedullary haematopoiesis in the liver. Six days after immunization, no changes in the sheep red blood cell IgM titres were found in male mice dosed with the test substance, whereas the sheep red blood cell titres were significantly lower in mice of the positive control group.

Under the study conditions utilized, no signs of immunotoxicity were observed following administration of saflufenacil to male C57BL/6J Rj mice. The oral administration of the positive control substance led to findings indicative of immunotoxicity, demonstrating the sensitivity of the assay (Buesen, 2010; Buesen et al., 2010).

(d) Mechanistic studies of effects on porphyrins

In a repeated-dose mechanistic toxicity study, saflufenacil (purity 94.2%) was administered in the diet to groups of Wistar rats (10 of each sex per group) at 0, 1, 5 or 25 ppm (equal to 0, 0.1, 0.4 and 2.0 mg/kg bw per day in males and 0, 0.1, 0.5 and 2.3 mg/kg bw per day in females, respectively) for an 8-week period. The diets were analysed for stability, homogeneity and concentration. The rats were examined for signs of toxicity and mortality twice a day during weekdays and once a day during weekends and holidays. Body weights and feed consumption were determined once a week. Blood and faeces from all rats were sampled after 1, 2, 4 and 8 weeks of saflufenacil treatment. Haematological examinations were performed, and total porphyrin concentrations in faeces were measured. At study termination, all rats were sacrificed under carbon dioxide anaesthesia and assessed for gross pathological changes.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable (104–114% of the nominal). There were no adverse effects of treatment on mortality, clinical observation, body weight, feed consumption or haematological parameters. Dietary administration of saflufenacil at 25 ppm caused an increase in porphyrin in faeces of male and female rats (Table 33), whereas saflufenacil at 5 ppm caused an increase in faecal porphyrin only in males. At 1 ppm, there were no effects on porphyrin excretion in the faeces.

The LOAEL was established based upon effects on porphyrin metabolism seen in males at 5 ppm (equal to 0.4 mg/kg bw per day) and in females at 25 ppm (equal to 2.3 mg/kg bw per day). The NOAEL was 1 ppm for males (equal to 0.1 mg/kg bw per day) and 5 ppm for females (equal to 0.5 mg/kg bw per day) (Cunha et al., 2005a).

In a repeated-dose mechanistic toxicity study, saflufenacil (purity 94.2%) was administered in the diet to groups of Wistar rats (10 of each sex per group) at 0, 10, 50 or 1000 ppm (equal to 0, 0.8, 4.1 and 80.6 mg/kg bw per day in males and 0, 0.9, 4.6 and 89.5 mg/kg bw per day in females, respectively) for an 8-week period. A 2-week recovery period was maintained for five animals of each sex per group. The effects of saflufenacil administration on porphyrin levels in plasma, urine, faeces and liver were monitored. The rats were examined for signs of toxicity and mortality twice a day during weekdays and once a day during weekends and holidays. Body weights and feed consumption were determined once a week. All surviving rats were sacrificed by carbon dioxide after a fasting period (withdrawal of feed and water) of about 16–20 hours. The liver was processed for porphyrin concentration examinations. No other examinations were carried out.

No analyses of the test substance preparations were carried out for this study. No treatment-related clinical signs or mortality occurred during the study. No treatment-related changes in body weight were observed in any of the treated animals. High-dose males had lower body weights in the first 2 weeks of the treatment; however, the overall weight gain of high-dose males was similar to that of control males. Feed consumption was found to be significantly reduced only in males treated with the high concentration (1000 ppm) on study days 7 (−9%) and 21 (−10%). At the end of the administration period, significantly decreased haemoglobin, haematocrit, MCV and MCH values were found in the peripheral blood of the male and female animals receiving 1000 ppm of the test compound. In addition, MCHC was significantly reduced in the males of the 1000 ppm group. No treatment-related effects on any of the haematological parameters were noted in either sex of the 10 or 50 ppm groups. At the end of the recovery period (week 11), high-dose rats still

Table 33. Total porphyrin values in faeces

	Mean total porphyrin values in faeces ± standard deviation (µmol/l)							
	Males (n = 10 per group)				Females (n = 10 per group)			
	Dietary concentration (ppm)							
	0	1	5	25	0	1	5	25
Day 7	76.1 ± 29.4	97.6 ± 43.5	**109 ± 40.4***	**283 ± 60.5****	103 ± 30.5	88.5 ± 40.6	109 ± 49.8	**175 ± 60.9****
Day 14	48.8 ± 31.5	46.3 ± 20.5	**75.9 ± 36.9***	**207 ± 74.0****	76.9 ± 54.7	70.9 ± 35.0	95.6 ± 38.7	**128 ± 44.1***
Day 28	57.2 ± 26.6	58.8 ± 26.8	**116 ± 56.8***	**204 ± 70.7****	71.2 ± 24.3	70.6 ± 32.9	87.5 ± 34.0	**145 ± 39.6****
Day 57	48.4 ± 19.6	58.2 ± 15.8	**110 ± 61.1***	**163 ± 57.0****	86.7 ± 23.4	109 ± 54.6	102 ± 40.3	140 ± 63.0

From Cunha et al. (2005a). Data taken from table IB, pp. 88–103 of the study report.
* $P \leq 0.05$; ** $P \leq 0.01$; **bolded** values are considered to be treatment related

showed statistically significantly decreased MCHC (males), MCV (females) and MCH (females) values when compared with the controls, but the differences were only marginal, indicating that the effects of saflufenacil on the blood parameters were reversible after a 2-week recovery period. Distinct increases in total porphyrin concentrations were measured in the plasma of the 1000 ppm groups of males (approximately 3- to 10-fold higher than the controls) and females (approximately 3-fold higher than the controls) after 3, 5 and 9 weeks of test substance administration (Table 34). In addition, porphyrin levels were slightly increased in the males of the 50 ppm group (approximately 2-fold higher than the controls) at weeks 3 and 5. Increases in total porphyrins were observed in the urine samples of the 1000 ppm groups of the male (approximately 4-fold higher than the controls) and female (approximately 5- to 8-fold higher than the controls) animals at the 3-, 5- and 9-week intervals. Slight, but statistically significant, increases in urinary total porphyrin concentrations were also found in the 50 ppm groups of both sexes (approximately 2-fold higher than the controls) after 3 and 9 weeks of test substance administration. No treatment-related effects on total porphyrin levels in urine of either sex were seen at 10 ppm. Throughout the administration period, statistically significantly increased total porphyrin concentrations were found in the faeces of the 50 ppm (males: approximately 3- to 7-fold higher; females: approximately 2- to 3-fold higher than the controls) and 1000 ppm (males: approximately 8- to 11-fold higher than the controls; females: approximately 7- to 11-fold higher than the controls) groups of both sexes. In the males, significantly increased porphyrin levels were also noted in the animals of the 10 ppm group (approximately 2-fold higher than the controls) at the 5- and 9-week intervals. No changes were seen in the females given 10 ppm of the test compound. After cessation of treatment, all test compound–related findings were reversible within a recovery period of 2 weeks. Statistically significant increases in porphyrin levels in liver homogenates were observed in rats at 10 ppm (females 1.5-fold higher than controls; statistically non-significant), 50 ppm (males approximately 5-fold and females approximately 3-fold higher than controls) and 1000 ppm (males approximately 27-fold and females approximately 24-fold higher than controls).

After 2 and 4 weeks of dietary exposure to saflufenacil, δ-aminolevulinic acid concentrations in urine samples of high-dose males were slightly, but statistically significantly, increased (approximately 3-fold higher than controls). After 2 and 4 weeks of dietary exposure to saflufenacil, increases of porphobilinogen concentrations were observed in high-dose males (approximately 8-fold higher than controls) and females (approximately 2-fold higher than controls at week 4). After 1, 2 and 4 weeks of dietary exposure to saflufenacil, dose-dependent increases in coproporphyrin III concentrations were observed in urine of treated rats. The concentration of coproporphyrin III was generally higher in male than in female animals. Significant increases of coproporphyrin III concentration in faeces were observed in all test rats at 1000 ppm (males, approximately 17-, 12- and 10-fold higher than controls on study days 7, 14 and 28, respectively; females, approximately 22-, 25- and 14-fold higher

Table 34. Porphyrin values in plasma, urine, faeces and liver

	Porphyrin values (mean ± standard deviation)							
	Males (*n* = 5 per group)				Females (*n* = 5 per group)			
	Dietary concentration (ppm)							
	0	10	50	1000	0	10	50	1000
Total porphyrin in plasma, nmol/l (*n* = 10 per group at week 9)								
Week 3	27.8 ± 9.3	44.6 ± 24.6	**69.1 ± 38****	**190 ± 119****	32.9 ± 8.6	24.5 ± 9.0	33.8 ± 11.7	**106 ± 16****
Week 5	21.8 ± 5.4	26.6 ± 7.5	**37.4 ± 9.1***	**219 ± 113****	23.8 ± 7.8	15.8 ± 3.5	24.7 ± 4.4	**76.1 ± 10****
Week 9	43.6 ± 20.5	53.2 ± 27.4	53.0 ± 17.1	**133.2 ± 88****	38.8 ± 23.6	27.8 ± 16.3	35.0 ± 14.3	**109 ± 138****
δ-Aminolevulinic acid in urine, µmol/l								
Week 3	48.6 ± 18.3	57.4 ± 9.1	54.7 ± 27.5	**119.8 ± 59***	62.1 ± 24.6	65.4 ± 34.1	49.9 ± 12.8	68.0 ± 27.4
Week 5	41.1 ± 20.4	45.2 ± 5.2	39.6 ± 8.4	**97.8 ± 26***	50.3 ± 17.9	67.5 ± 31.7	44.6 ± 7.6	56.7 ± 7.7
Porphobilinogen in urine in urine, µmol/l								
Week 3	4.8 ± 0.5	5.0 ± 1.5	6.1 ± 1.3	**35.9 ± 22****	5.1 ± 1.1	6.1 ± 2.7	4.3 ± 0.9	**9.8 ± 2.22****
Week 5	5.6 ± 0.6	6.2 ± 1.4	5.6 ± 0.6	**39.3 ± 20****	5.0 ± 0.9	5.8 ± 0.8	4.9 ± 0.9	7.3 ± 2.1
Total porphyrin in urine, µmol/l (*n* = 10 per group at week 9)								
Week 3	3.4 ± 1.9	5.3 ± 2.7	**8.6 ± 1.9****	**15.4 ± 2.1****	1.3 ± 0.3	1.2 ± 0.3	2.8 ± 1.2*	**10.3 ± 3.6****
Week 5	3.3 ± 2.1	3.8 ± 1.9	5.9 ± 0.7	**14.0 ± 1.3****	1.6 ± 0.8	1.1 ± 0.3	2.1 ± 0.5	**7.5 ± 3.0****
Week 9	3.8 ± 2.3	3.7 ± 1.8	**6.7 ± 1.9***	**17.2 ± 4.6****	1.1 ± 0.4	1.1 ± 0.3	2.4 ± 1.0**	**10.4 ± 5.3****
Total porphyrin in faeces, µmol/l (*n* = 10 per group at week 9)								
Week 3	74.0 ± 39.5	150.7 ± 68	**447 ± 127****	**615 ± 247****	67.0 ± 10.4	126.9 ± 104	**220 ± 139****	**615 ± 247****
Week 5	47.0 ± 2.9	**123.6 ± 61***	**328 ± 174****	**539 ± 203****	97.4 ± 40.3	83.9 ± 29.1	**183 ± 50***	**539 ± 203****
Week 9	63.8 ± 33.5	**106.4 ± 46***	**217 ± 102****	**618 ± 155****	89.8 ± 50.9	112.9 ± 53	**176 ± 75****	**635 ± 209****
Week 11	90.3 ± 63.3	40.0 ± 15	53.3 ± 39.9	54.1 ± 19.3	81.0 ± 63.9	74.6 ± 39.7	53.3 ± 39.9	64.3 ± 25.9
Total porphyrin in liver, pmol/l								
Week 9	96.6 ± 39.7	175.6 ± 45	**504 ± 79****	**2572 ± 489****	115 ± 28.2	196 ± 42	**389 ± 80****	**2753 ± 856****

From Cunha et al. (2006). Data taken from table IB, pp. 88–103 of the study report.
* $P \leq 0.05$; ** $P \leq 0.01$; **bolded** values are considered to be treatment related

than controls on study days 7, 14 and 29, respectively), 50 ppm (males, approximately 6-, 6- and 4-fold higher than controls on study days 7, 14 and 28, respectively; females, approximately 3-fold higher than controls on study days 7, 14 and 29) and 10 ppm (males, approximately 2-fold higher than controls on study days 7 and 14; females, approximately 1.5-fold higher than controls on study days 7 and 29). There were no pronounced increases of protoporphyrin IX in faeces of both male and female rats. Significant increases in the concentration of mesoporphyrin in faeces were found at 1000 ppm and 50 ppm in males and females and also at 10 ppm in males only. Comparison of the specific (HPLC) and nonspecific (spectrofluorometric) methods of determination of porphyrin excretion in urine and faeces indicated that the total values obtained from both methods were similar. There were no treatment-related gross pathological findings.

In summary, dietary administration of saflufenacil at 1000 ppm caused moderate MHA (sideroblastic anaemia) due to inhibition of haem biosynthesis. The inhibition of PPO resulted in increased accumulation and excretion of large amounts of porphyrins and their precursors, resulting in marked porphyria. Animals treated with lower concentrations of saflufenacil showed no signs of anaemia. At 1000 ppm, anaemia was observed in both sexes. However, at 50 ppm, saflufenacil still caused significant porphyria in male and female rats. Small, but statistically significant, increases in porphyrin levels in urine of males and in faeces of both sexes at 10 ppm were still noted. In general,

the total porphyrin concentrations in the plasma, urine, faeces and liver were statistically significantly increased in male and female rats at 50 and 1000 ppm. Following a 2-week recovery period, total porphyrins in the faeces of both sexes and most haematological parameters returned to normal or showed trends of reversibility. Therefore, it was concluded that the inhibition of PPO by saflufenacil was rapidly and completely reversible after cessation of treatment. Both individual porphyrin (HPLC) and total porphyrin (spectrofluorometric) measurements were made to assess the correlation of the two methods. The results indicated a strong correlation, and both types of measurements provided reliable dose–response information regarding PPO inhibition. Total porphyrins in faeces and liver provided the most reliable and sensitive data. Statistically significant effects on porphyrin metabolism could be detected at exposure concentrations well below those associated with adverse haematological effects.

Effects on porphyrins were seen down to the lowest dose tested, giving a lowest-observed-effect level (LOEL) of 10 ppm (equal to 0.8 mg/kg bw per day) and a corresponding no-observed-effect level (NOEL) of less than 10 ppm (equal to 4.1 mg/kg bw per day) (Cunha et al., 2006).

(e) Comparative study of hydrate and anhydrate forms of saflufenacil

Saflufenacil hydrate (purity 93.9%) or saflufenacil anhydrate (purity 99.0%) was administered to groups of 10 male Wistar rats at a dietary concentration of 0 or 1350 ppm (equal to 126.0–129.1 mg/kg bw per day) over a period of 4 weeks in order to determine if there are toxicologically significant differences between the two different crystalline forms of saflufenacil. After dosing, the rats were examined for signs of toxicity or mortality twice a day. Body weights were determined on day 0, then weekly thereafter. Feed consumption was recorded weekly. Faeces and blood from all rats were sampled on days 14 and 28 for haematological and porphyrin determinations. At study termination, all rats were sacrificed under carbon dioxide anaesthesia and assessed for gross pathological changes. The weights of the spleen and liver were recorded. The livers were also analysed for porphyrin concentrations.

The results indicated very similar effects following dietary exposure to the hydrated or anhydrate form of saflufenacil. The effects included impairment of feed intake; significant decreases in erythrocyte counts, haemoglobin, haematocrit, MCV, MCH, MCHC; increased reticulocyte and platelet counts; increases in porphyrin levels in the liver and faeces; and increases in absolute and relative spleen weights. Based on the similarity of the findings, it was concluded that the bioavailability and toxicity potential of the hydrated and anhydrated form of saflufenacil were similar (Cunha et al., 2005b).

(f) Dermal absorption

Rats

The absorption, distribution and excretion of radiolabelled saflufenacil were studied in male Wistar rats following a single dermal application of [^{14}C]saflufenacil (radiochemical purity 95%) suspended in the formulation concentrate (BAS 800 02 H) and 1/10 and 1/100 aqueous dilutions thereof. The nominal dose levels were 1.1723 mg/cm^2, 0.1172 mg/cm^2 and 0.0117 mg/cm^2, which correspond to about 11.723, 1.172 and 0.117 mg/animal at the high, middle and low doses, respectively. The high, middle and low doses selected in this study correspond to approximately 36.9, 4.0 and 0.4 mg/kg bw. Hairs were clipped from the dorsal region 24 hours before treatment, and the region was then cleaned with acetone. All animals were exposed for 10 hours. Application sites (all dose groups) were washed at 10 hours with a mild soap solution. Four animals were killed at 10, 24, 72 and 120 hours post-application. Radioactivity in the carcass, urine, faeces, blood, treated skin (after washing), surrounding skin, cage wash, gauge and bandages was analysed.

Mean recoveries of radioactivity from all dose groups were in the range of 92.00–115.20% of the total radioactivity administered. The largest proportion of radioactivity was recovered from the carcass and the faecal samples from the high-dose group and in the skin washes of the mid-dose (about 82–93% of the applied dose) and low-dose groups (about 72–86% of the applied dose). At the

high dose (i.e. the formulation concentrate), a systemic absorption of about 66.41% was observed after a 10-hour period of exposure to saflufenacil. At sacrifice after 120 hours, the absorption was 81.01% of the dose. In the high-dose group, about two thirds of the radioactivity remaining in the skin after the end of exposure penetrated through the skin during the 5-day post-observation period. At the middle dose (i.e. an 1/10 aqueous dilution of the formulation concentrate), a systemic absorption of about 2.78% was observed immediately after a 10-hour exposure period to saflufenacil. At sacrifice after 24, 72 and 120 hours, the absorptions were 3.96%, 8.57% and 3.36%, respectively. At the low dose (i.e. a 1/100 aqueous dilution of the formula concentrate), a systemic absorption of about 3.39% was observed immediately after a 10-hour exposure period for saflufenacil. At sacrifice after 24, 72 and 120 hours, the absorptions were 4.80%, 4.01% and 5.94% of the radioactivity applied, respectively. In most dermal absorption studies, the highest per cent absorption is usually observed at the low doses, whereas in this study, the highest dermal absorption was at the highest dose. There was evidence of skin irritation/corrosion at the application site in the high dose, which may enhance the dermal absorption. About 21–30% of the radioactivity was bound to the protective device in the high-dose group compared with 1.2–8.26% at the mid- and low-dose groups, suggesting the potential for even higher absorption potential at the high dose if the protective device–bound material was available for absorption (Fabian & Landsiedel, 2007b).

To estimate the dermal absorption of saflufenacil simulating the user-specific exposure scenario, the absorption, distribution and excretion of radiolabelled saflufenacil were studied in male Wistar rats following a single dermal application of saflufenacil in the formulation concentrate (BAS 800 04H) and 1/10 and 1/100 aqueous dilutions thereof. The target dose levels were 3.42 mg/cm^2, 0.342 mg/cm^2 and 0.0342 mg/cm^2 (corresponding to about 34.2, 3.42 and 0.342 mg/animal and about 105.8, 11.2 and 1.06 mg/kg bw) at the high, middle and low doses, respectively. Skin wash was performed at 8 hours after exposure and also before sacrifice at each time point. Four treated rats per dose were sacrificed at 24, 72 and 168 hours post-exposure. Excreta (urine and faeces) were collected throughout the exposure period, and blood cells, plasma, treated skin, skin surrounding the treated area and the carcass were collected at sacrifice. In addition, the cage and skin washes as well as the protective cover were retained for the determination of radioactivity.

There was no skin irritation observed at the skin application site. Mean recoveries of radioactivity from all dose groups ranged from 95.27% to 107.6% of the applied dose. The radioactivity in skin wash ranged from about 78% to 99% of the applied dose. Mean dermal absorption for saflufenacil in the BAS 800 04 H formulation concentrate (3.42 mg/cm^2), 1/10 dilution of the concentrate (0.342 mg/cm^2) and 1/100 dilution of the concentrate (0.0342 mg/cm^2) was 0.32%, 0.56% and 1.63%, respectively, following an exposure period of 8 hours and sacrifice after 168 hours. This study (Fabian & Landsiedel, 2008) gave drastically reduced dermal absorption values compared with the previous study (Fabian & Landsiedel, 2007b), probably due to differences in the vehicle used in these two studies.

(g) Protoporphyrinogen IX oxidase in vitro

A non-guideline study was conducted to investigate the inhibitory effects of saflufenacil (purity 93.8%) on PPO in liver mitochondrial preparations from female Wistar rats, female C57BL/6NCrl mice, female Himalayan rabbits and female human donors. The porphyrin pathway is an essential metabolic pathway in haem and chlorophyll biosynthesis. PPO catalyses the oxygen-dependent oxidation of protoporphyrinogen IX to protoporphyrin IX. In plants, the porphyrin pathway is a target for herbicides and growth-regulating chemicals. In mammals, inhibition of PPO may induce toxic effects. PPO is localized in two different compartments: in plants, PPO is localized in chloroplasts and in mitochondria; in mammals, PPO is localized in mitochondria. Mitochondrial fractions of female Wistar rats, C57BL/6N Crl mice, Himalayan rabbits and human donors were prepared and characterized

according to the activity of marker enzymes. Measurements of PPO activities of liver mitochondrial preparations were based on the spectrofluorometric detection of protoporphyrin formation over time. Butafenacil and oxyfluorfen (known PPO inhibitors) were used as positive controls.

PPO activities were linear to the amount of mitochondrial homogenate and varied across species, with human mitochondrial fractions showing lower PPO activity per milligram of protein, followed by the rabbit, mouse and rat. The rate of inhibition was determined using similar amounts of protein per assay, following the addition of various concentrations of saflufenacil. Butafenacil and oxyfluorfen, known PPO inhibitors, were similarly used as positive controls for PPO activity inhibition. PPO inhibition values were used for the determination of the 50% inhibitory concentrations (IC_{50}s) for interspecies comparison of inhibitory potency. The results demonstrated interspecies differences in saflufenacil inhibition of PPO activities in rat, mouse, rabbit and human liver mitochondrial preparations.

The relative inhibitory potency of saflufenacil on mice hepatic mitochondria was 0.6 times higher when compared with what was observed in rat mitochondria. The greatest differences in mitochondrial inhibition between species were observed in humans (14.1 times) and rabbits (16.2 times), when compared with inhibitory effects on rats, meaning that humans are approximately 14 times less sensitive than rats to PPO inhibition. No statistically significant differences were observed between human and rabbit mitochondria. The inhibitory activity of the other PPO inhibitors tested (butafenacil and oxyfluorfen) in different species was similar to that observed for saflufenacil; however, saflufenacil showed the lowest overall inhibition of PPO enzyme activity (Fabian, Niggeweg & Landsiedel, 2008).

3. Observations in humans

No clinical cases or poisoning incidents relating to saflufenacil have been reported.

Comments

Biochemical aspects

Absorption, distribution, excretion and metabolism of orally administered (gavage) saflufenacil were studied in male and female rats using (phenyl-U-^{14}C)- and (uracil-4-^{14}C)-labelled saflufenacil. The time to reach the maximum concentration of radioactive material in plasma was less than 1 hour. Thereafter, the plasma level of radioactivity declined rapidly, and only residual radioactivity was detected at 168 hours (0.2% of the administered dose). The AUC values indicated a sex difference, with up to 3-fold higher internal exposures for males than for females. Saflufenacil was rapidly and extensively (> 79%) absorbed from the gastrointestinal tract and rapidly excreted from the body in urine and faeces (> 97% of the administered dose) within 168 hours. The majority of excretion occurred in the first 24–48 hours, and excretion was complete by 96 hours. In 48 hours, bile duct–cannulated rats excreted approximately 67.8% and 35.5% of the administered dose in the bile in males and females, respectively. The urinary and biliary excretion data suggested that significant enterohepatic circulation of saflufenacil had occurred. Within 1 hour after oral administration of [^{14}C]saflufenacil, the highest radioactivity was found in the liver, gastrointestinal tract, kidney, lung and thyroid.

In the urine, the unchanged parent compound accounted for 10.9–48.2% and 78.1–88.9% of the administered dose for male and female rats, respectively. The predominant metabolic reactions of saflufenacil in the rat were demethylation of the uracil ring system, stepwise degradation of the *N*-methyl-*N*-isopropylsulfonamide to form an unsubstituted sulfonamide and cleavage of the uracil ring with loss of a three-carbon fragment to form an *N*-methylurea attached to the phenyl ring. The major metabolites identified in the urine of male and female rats were M800H01 (3.5–9.1% of the

dose) and M800H07 (0.6–4.6% of the dose), respectively. In faeces, the parent compound accounted for 3–16% of the dose. The main metabolite in faeces was M800H01, which amounted to 18–44% and 1–3% of the dose in male and female rats, respectively.

Toxicological data

The LD_{50} in rats treated orally and dermally with saflufenacil was greater than 2000 mg/kg bw. The LC_{50} in rats treated by inhalation (nose only) was greater than 5.3 mg/l. Saflufenacil was minimally irritating to the eyes and non-irritating to the skin of rabbits. Saflufenacil was not a skin sensitizer in guinea-pigs, as determined by the Magnusson and Kligman (maximization) test.

Short-term toxicity studies in mice, rats and dogs showed similar profiles of toxicity with respect to blood and liver. Males were more susceptible than females. The haematological effects were mostly related to the pesticidal mode of action of saflufenacil (i.e. inhibition of PPO). Effects indicative of this included increased total porphyrins in urine, faeces and liver, as well as increased total bilirubin and urinary bilinogen. Decreased haematological parameters indicative of MHA are consistent with this mode of action. Indicators of MHA included increased normoblasts, reticulocytes and polychromasia, increased microcytosis and anisocytosis, increased spleen weight, extramedullary haematopoiesis in liver and spleen (iron storage) and erythroid hyperplasia in bone marrow. At higher doses, an indication of liver toxicity, which included increased serum liver enzymes, centrilobular fatty change and lymphoid cell infiltration, was observed.

In 28-day and 90-day toxicity studies in mice, MHA, altered clinical chemistry (increased ALT, AST, urea and total bilirubin) (28-day study) and liver pathology (increased weight and centrilobular fatty change) were observed. In addition, decreased body weight and body weight gain were observed in the 90-day toxicity study. The NOAEL in the 28-day and 90-day studies of toxicity in mice was 50 ppm (equal to 12.8 mg/kg bw per day). The LOAEL in the 28-day and 90-day toxicity studies in mice was 150 ppm (equal to 36.6 mg/kg bw per day).

In a 28-day toxicity study in rats, the NOAEL was 150 ppm (equal to 13.4 mg/kg bw per day), based on MHA at 450 ppm (equal to 39.2 mg/kg bw per day). In addition to MHA, decreased total protein and decreased globulin were observed in a 90-day toxicity study in rats. The NOAEL in the 90-day toxicity study was 150 ppm (equal to 10.5 mg/kg bw per day), and the LOAEL was 450 ppm (equal to 32.3 mg/kg bw per day).

In a 28-day toxicity study in dogs, the NOAEL was 30 mg/kg bw per day, based on MHA at 100 mg/kg bw per day. The NOAEL in a 90-day toxicity study in dogs was 10 mg/kg bw per day, based on MHA in both sexes at 50 mg/kg bw per day. At the highest dose tested, more severe anaemia was seen, along with decreased body weight and body weight gain and dark brown/red brown discoloured faeces. In a 1-year toxicity study in dogs, the NOAEL was 20 mg/kg bw per day, based on discoloured faeces, lower body weight in males, decreased feed consumption, MHA, increased serum alkaline phosphatase activity and lowered total blood protein and albumin levels at 80 mg/kg bw per day. The overall NOAEL for the 90-day and 1-year toxicity studies in dogs was 20 mg/kg bw per day.

The carcinogenic potential of saflufenacil was studied in mice and rats. In mice, there was unusually high mortality in controls and all dose groups after approximately 16 months (485 days) of treatment. However, survival was adequate to assess the carcinogenic potential of saflufenacil. The early mortality was greatest in the control and low-dose male mice and was clearly unrelated to test substance treatment. There were no treatment-related effects on clinical signs of toxicity, mortality, body weight and body weight gain, feed consumption and feed efficiency, gross pathology or organ weights. The NOAEL was 25 ppm (equal to 4.6 mg/kg bw per day), based on MHA seen in the satellite group (killed at 10 months) at 75 and 150 ppm (equal to 13.8 and 38.1 mg/kg bw per day) in males and females, respectively. No treatment-related tumours were observed in mice.

In a 2-year study of toxicity and carcinogenicity in rats, the NOAEL was 100 ppm (equal to 6.2 mg/kg bw per day), on the basis of decreased body weight and body weight gains (males), anogenital region smeared with urine in females and MHA in males and females at 500 ppm (equal to 24.2 mg/kg bw per day). No treatment-related tumours were observed in rats.

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

Saflufenacil gave a negative response in an adequate range of in vitro and in vivo genotoxicity tests, except for a positive finding that occurred with metabolic activation in an in vitro chromosomal aberration assay in mammalian cells. In contrast, no clastogenicity was observed in an in vivo mouse micronucleus assay.

The Meeting concluded that saflufenacil was unlikely to be genotoxic in vivo.

On the basis of the absence of genotoxicity in vivo and the absence of carcinogenicity in mice and rats, the Meeting concluded that saflufenacil is unlikely to pose a carcinogenic risk to humans.

In a two-generation study of reproductive toxicity in rats, reproductive parameters were not affected at the highest dose tested (50 mg/kg bw per day). The NOAEL for parental systemic toxicity was 15 mg/kg bw per day, based on adverse effects on feed intake, body weight gain and MHA at 50 mg/kg bw per day. The NOAEL for offspring toxicity was 15 mg/kg bw per day, based on the increased number of stillborn pups, increased pup mortality during the early phase of lactation, reduced pup weight gains and indications of MHA at 50 mg/kg bw per day.

In a developmental toxicity study in rats, the NOAEL for maternal toxicity was 20 mg/kg bw per day, based on MHA at 60 mg/kg bw per day. The developmental toxicity NOAEL was 5 mg/kg bw per day, based on decreased fetal body weights in males and females, an increased incidence of skeletal anomalies and delayed ossification at 20 mg/kg bw per day. In a developmental toxicity study in rabbits, the NOAEL for maternal toxicity was 200 mg/kg bw per day, based on increased mortality, clinical signs (lateral positioning, poor general state, abortion, blood in bedding, discoloured and no urination and reduced or no defecation) and increased necropsy findings (stomach ulcerations, lack of faeces, increase in pale livers and kidneys, empty stomachs, enlarged bladders and assorted findings on implantations in dams that aborted or were moribund) at 600 mg/kg bw per day. The developmental toxicity NOAEL was 200 mg/kg bw per day, based on a decrease in total litters and total live fetuses and live fetuses per dam at 600 mg/kg bw per day.

The Meeting concluded that saflufenacil is not teratogenic in rats or rabbits.

In an acute neurotoxicity study in rats via gavage, no effects on FOB parameters, motor activity or neuropathology were observed at doses up to 2000 mg/kg bw. For systemic toxicity, the NOAEL was 5000 mg/kg bw for male rats, based on the decreased motor activity, representing mild and transient systemic toxicity, likely due to general malaise, at 2000 mg/kg bw, the highest dose tested.

In a 90-day dietary study of neurotoxicity in rats, no effects on FOB parameters, motor activity or neuropathology were observed in males and females at doses up to 1000 and 1350 ppm, respectively (equal to 66.2 and 101.0 mg/kg bw per day for male and female rats, respectively). The NOAEL for systemic toxicity was 250 ppm (equal to 16.6 mg/kg bw per day), based on MHA at 1000 ppm (equal to 66.2 mg/kg bw per day).

In an immunotoxicity study, no evidence of immunotoxicity was observed in male mice treated with saflufenacil in the diet for 4 weeks at doses up to 250 ppm (equal to 52 mg/kg bw per day).

Two dietary toxicity studies were conducted in rats to evaluate the effects of saflufenacil administration on porphyrin levels in plasma, urine, faeces and liver and also to evaluate the reversibility of porphyrin levels. Total porphyrin measurements showed significantly higher total porphyrin levels in the faeces of the males at 5 and 25 ppm (equivalent to 0.4 and 2.0 mg/kg bw per day, respectively) and of the females at 25 ppm (equivalent to 2.3 mg/kg bw per day). These findings are considered to be treatment related and are a consequence of increased accumulation and excretion of porphyrins due to inhibition of PPO by saflufenacil. In the recovery study, during a

treatment-free recovery period of 2 weeks, the statistically significant increases in total porphyrins in the faeces of both sexes returned to normal. Most of the haematological effects indicated their complete reversibility.

In studies in rats, bioavailability and toxicity were comparable between hydrated and anhydrate crystalline forms of saflufenacil.

An in vitro study was conducted to investigate the inhibitory effects of saflufenacil on PPO in the liver mitochondrial preparations from female rats, mice, rabbits and human donors. The results of this study indicated that rats are approximately 14 and 16 times more susceptible than humans and rabbits, respectively, to PPO inhibition. This difference in species susceptibility is consistent with the absence of haematological effects in rabbits at doses at least 14 times the NOAEL for these effects in rats.

No adverse effects due to occupational exposure to saflufenacil were reported in employees having contact with the active substance.

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

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) of 0–0.05 mg/kg bw on the basis of a NOAEL of 4.6 mg/kg bw per day in the carcinogenicity study in mice, based on MHA at 13.8 mg/kg bw per day, and using a safety factor of 100. This ADI was supported by the NOAEL of 6.2 mg/kg bw per day observed in the chronic toxicity and carcinogenicity study in rats, on the basis of anogenital region smeared with urine in female rats and MHA in rats seen at 24.2 mg/kg bw per day. It is further supported by the NOAEL of 5 mg/kg bw per day observed in the developmental toxicity study in rats on the basis of increased skeletal anomalies at 20 mg/kg bw per day.

The Meeting concluded that it was not necessary to establish an acute reference dose (ARfD) for saflufenacil in view of its low acute toxicity and the absence of developmental toxicity or any other toxicological effects that would be likely to be elicited by a single dose. MHA is not considered to be an appropriate end-point on the basis of which to establish an ARfD because it is not expected to appear after a single exposure due to the mechanism of toxicity by which it is produced.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Sixteen-month study of toxicity and carcinogenicity[a]	Toxicity	25 ppm, equal to 4.6 mg/kg bw per day	75 ppm, equal to 13.8 mg/kg bw per day
		Carcinogenicity	75 ppm, equal to 13.8 mg/kg bw per day[b]	—
Rat	Two-year study of toxicity and carcinogenicity[a]	Toxicity	100 ppm, equal to 6.2 mg/kg bw per day	500 ppm, equal to 24.2 mg/kg bw per day
		Carcinogenicity	500 ppm, equal to 24.2 mg/kg bw per day[b]	—
	Two-generation study of reproductive toxicity[a]	Parental toxicity	15 mg/kg bw per day	50 mg/kg bw per day
		Reproductive toxicity	50 mg/kg bw per day[b]	—
		Offspring toxicity	15 mg/kg bw per day	50 mg/kg bw per day
	Developmental toxicity study[c]	Maternal toxicity	20 mg/kg bw per day	60 mg/kg bw per day
		Embryo and fetal toxicity	5 mg/kg bw per day	20 mg/kg bw per day

Species	Study	Effect	NOAEL	LOAEL
	Acute neurotoxicity study[c]	Systemic toxicity	500 mg/kg bw	2000 mg/kg bw
		Neurotoxicity	2000 mg/kg bw[b]	—
Rabbit	Developmental toxicity study[c]	Maternal toxicity	200 mg/kg bw per day	600 mg/kg bw per day
		Embryo and fetal toxicity	200 mg/kg bw per day	600 mg/kg bw per day
Dog	Ninety-day and 1-year studies of toxicity[a,d]	Toxicity	20 mg/kg bw per day	50 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

0–0.05 mg/kg bw

Estimate of acute reference dose

Not necessary

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 saflufenacil

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapidly absorbed, complete within 168 h
Dermal absorption	No data available
Distribution	Widely distributed in tissues; highest residues in liver, gastrointestinal tract, liver, kidney, lung and thyroid
Potential for accumulation	None
Rate and extent of excretion	Rapid and extensive
Metabolism in animals	Moderately metabolized
Toxicologically significant compounds (animals, plants and environment)	Saflufenacil
Acute toxicity	
Rat, LD_{50}, oral	> 2000 mg/kg bw (female rats)
Rat, LD_{50}, dermal	> 2000 mg/kg bw
Rat, LC_{50}, inhalation	> 5.3 mg/l (4 h exposure, nose only)
Rabbit, dermal irritation	Non-irritating
Rabbit, ocular irritation	Minimally irritating
Guinea-pig, dermal sensitization (Magnusson and Kligman test)	Not a sensitizer
Short-term studies of toxicity	
Target/critical effect	MHA (mice, rats and dogs)
Lowest relevant oral NOAEL	10.5 mg/kg bw per day (90-day study of toxicity in rats)
Lowest relevant dermal NOAEL	1000 mg/kg bw per day (rats)
Lowest relevant inhalation NOAEC	Not available

Long-term studies of toxicity and carcinogenicity	
Target/critical effect	MHA
Lowest relevant NOAEL	4.6 mg/kg bw per day (carcinogenicity study in mice)
Carcinogenicity	Not carcinogenic in mice and rats
Genotoxicity	
	Not genotoxic in vivo
Reproductive toxicity	
Reproduction target/critical effect	None
Lowest relevant reproductive NOAEL	50 mg/kg bw per day (rats; highest dose tested)
Developmental target/critical effect	Developmental toxicity, including skeletal anomalies in rats
Lowest relevant developmental NOAEL	5 mg/kg bw per day (rats)
Neurotoxicity/delayed neurotoxicity	
Neurotoxicity target/critical effect	Not neurotoxic (acute and 90-day studies in rats)
Lowest relevant neurotoxicity NOAEL	66.2 mg/kg bw per day, highest dose tested
Mechanistic data	
	Mechanistic studies indicating species differences in PPO inhibition and reversibility of porphyria
Medical data	
	No adverse effects reported

Summary

	Value	Study	Safety factor
ADI	0–0.05 mg/kg bw	Carcinogenicity study in mice supported by 2-year study of toxicity and carcinogenicity in rats and developmental toxicity study in rats	100
ARfD	None established		

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SULFOXAFLOR

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Explanation

Sulfoxaflor is the International Organization for Standardization (ISO)–approved name for [methyl(oxo){1-[6-(trifluoromethyl)-3-pyridyl]ethyl}-λ^6-sulfanylidene]cyanamide (International Union of Pure and Applied Chemistry [IUPAC]) (Chemical Abstracts Service No. 946578-00-3), a novel insecticide from the sulfoximine class. Sulfoxaflor contains two chiral centres (the sulfur atom and the

carbon atom attached to position 3 of the pyridine ring) and is a mixture of the four possible stereoisomers. Both (*E*)- and (*Z*)-isomers (involving the S=N double bond and the cyano group) exist, but they rapidly interconvert at ambient temperatures. Sulfoxaflor is effective against a wide range of sap-feeding insects and exerts its insecticidal activity as an agonist at the insect nicotinic acetylcholine receptor (nAChR), which plays a central role in the mediation of fast excitatory synaptic transmission in the insect central nervous system. Sulfoxaflor 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.

All pivotal studies were certified as complying with good laboratory practice (GLP) or an approved quality assurance programme.

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 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 purity of the test substance used in the majority of toxicological studies was determined to be at least 95.6% weight per weight (w/w), comprising the (*E*)- and (*Z*)-diastereomers in a 50.5:49.5 ratio, by high-performance liquid chromatography.

1. Biochemical aspects

1.1 Absorption, distribution and excretion

In a pilot study conducted to determine the absorption, distribution, metabolism and elimination of sulfoxaflor in F344/DuCrl rats and Crl:CD1(ICR) mice, one male and one female rat and two male and two female mice received ^{14}C-labelled sulfoxaflor (purity 98.3%; radiochemical purity > 97%) as a 0.5% methylcellulose suspension by oral gavage at a single dose of 100 mg/kg body weight (bw); the dose volume was 5 ml/kg bw. Time-course blood samples (at 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48 and 72 hours post-dosing) and excreta were collected from rats, whereas only excreta were collected from mice up to 72 hours post-administration. The urine samples collected during the first 12 hours post-dosing were analysed for parent compound and metabolites. Additionally, two rats (one male, one female) and four mice (two males, two females) were orally dosed with ^{14}C-labelled sulfoxaflor, and plasma was collected at the time of peak concentration, or C_{max} (T_{max}: 2 hours post-dosing for rats and 1.5 hours post-dosing for mice), and analysed for parent and metabolites.

The orally administered ^{14}C-labelled sulfoxaflor was rapidly absorbed from the gastrointestinal tract in both rats and mice. The plasma C_{max} of radiolabelled material in male and female rats was 64 and 66 µg/g, respectively, which was reached 2 hours after the administration of the dose (Table 1). The C_{max} for red blood cells was 57 and 60 µg/g in male and female rats, which was reached in 1 and 2 hours, respectively. Elimination of radioactivity from plasma and red blood cells was slightly slower from male rats than from female rats, corresponding to a half-life during the terminal β phase ($t_{½β}$) of 9 and 11 hours (male) and 7 and 8 hours (female) for plasma and red blood cells, respectively. Faster elimination of the radioactivity by female rats was also reflected in 1.2-fold greater total body clearance (Cl), which was 129 and 141 ml/kg bw per hour from male plasma and red blood cells and 159 and 175 ml/kg bw per hour from female plasma and red blood cells, respectively. Similarly, the mean

Table 1. Summary of toxicokinetic parameters in the plasma of rats following oral administration of ^{14}C-labelled sulfoxaflor at 100 mg/kg bw

Parameter	Males	Females
T_{max} (h)	2	2
C_{max} (µg/g)	63.6	66.1
$t_{½β}$ (h)	8.8	7.2
$AUC_{0\rightarrow t}$ (µg·h/g)	817	611
$AUC_{0\rightarrow\infty}$ (µg·h/g)	819	663
Cl (ml/kg bw per hour)	129	159
MRT (h)	12.7	10.4

From Saghir, Clark & McClymont (2008)
AUC, area under the curve; Cl, clearance; C_{max}, peak concentration; MRT, mean residence time; $t_{½β}$, half-life during the terminal β phase; T_{max}, time to reach C_{max}

Table 2. Recovery of radioactivity in excreta and blood in rats and mice 72 hours post-dosing with ^{14}C-labelled sulfoxaflor at 100 mg/kg bw

	Recovery of radioactivity (% of administered dose)			
	Rats		Mice	
	Males	Females	Males	Females
Expired carbon dioxide	0.02	0.04	ND	ND
Plasma/red blood cells	0	0	0	0
Urine/rinse	98.05	87.24	84.69	79.80
Faeces	4.62	4.89	12.60	13.00
Total	102.69	92.16	97.29	92.80

From Saghir, Clark & McClymont (2008)
ND, not determined

residence time (MRT) of ^{14}C-labelled sulfoxaflor in the male rat was between 2 and 5 hours longer than in the female rat. The slower elimination and longer MRT of ^{14}C-labelled sulfoxaflor in the male rat resulted in 1.2-fold greater areas under the curve ($AUC_{0\rightarrow\infty}$: 819 and 663 µg·h/g for plasma and 751 and 603 µg·h/g for red blood cells).

Urinary elimination was 57–68% and 27–50% within 12 hours after dosing and increased to 77–83% and 66–70% of the administered dose within 24 hours for rats and mice, respectively. In total, 87–98% and 80–85% of the administered ^{14}C-labelled sulfoxaflor were recovered in the urine of rats and mice, respectively, within 72 hours post-dosing (Table 2). Faecal elimination accounted for only 5% in rats and 13% in mice, most likely representing unabsorbed sulfoxaflor, due to its recovery in faeces within the gastrointestinal transit time of 24 hours.

No metabolites of the test material were found in plasma following a single oral dose of ^{14}C-labelled sulfoxaflor to rats or mice, whereas trace levels of the urea metabolite (X11719474) were found in urine samples at levels comparable with its concentration as an impurity in the test material (≤ 0.44% of parent) (Saghir, Clark & McClymont, 2008).

In a study on toxicokinetics and metabolism conducted according to OECD test guideline 417, groups of four male and four female F344/DuCrl rats received sulfoxaflor-2-^{14}C-pyridine (purity 95.6%; radiochemical purity 99.7%) as a 0.5% methylcellulose suspension by oral gavage at a single dose of 5 or 100 mg/kg bw (groups 1 and 2) or repeated doses of 5 mg/kg bw (group 3; 14 daily doses

Table 3. Summary of toxicokinetic parameters in the plasma of rats following oral or intravenous administration of ^{14}C-labelled sulfoxaflor

Parameter	5 mg/kg bw, oral		100 mg/kg bw, oral		5 mg/kg bw, intravenous	
	Males	Females	Males	Females	Males	Females
T_{max} (h)	1.6	0.5	2.3	1.3	—	—
C_{max} (µg/g)	4.7	5.34	84.3	89.8	—	—
Absorption $t_{½}$ (h)	0.38	0.18	0.47	0.69	—	—
Elimination $t_{½\alpha}$ (h)	5.1	4.6	5.9	4.2	5.3	4.6
Elimination $t_{½\beta}$ (h)	39.1	39.6	39.4	45.0	42.3	44.1
$AUC_{0\rightarrow t}$ (µg·h/g)	48.7	44.7	1007	888	39.6	35.7
Cl (ml/kg bw per hour)	—	—	—	—	102	107

From Hansen et al. (2009)
AUC, area under the curve; Cl, clearance; C_{max}, peak concentration; $t_{½\alpha}$, half-life during the α elimination phase; $t_{½\beta}$, half-life during the terminal β phase; T_{max}, time to reach C_{max}

of non-radiolabelled sulfoxaflor followed by a single dose of ^{14}C-labelled sulfoxaflor on day 15); the dose volume was 5 ml/kg bw. In addition, four rats of each sex received ^{14}C-labelled sulfoxaflor as Intralipid 10% IV Fat Emulsion at a single intravenous dose of 5 mg/kg bw (group 4); the dose volume was about 2.5 ml/kg bw. The study continued for 168 hours post-dosing. Excreta and tissues were collected for groups 1–4, whereas time-course blood samples (at 0.25, 0.5, 1, 2, 4, 8, 12 and 24 hours and every 24 hours thereafter) were collected from animals of groups 1, 2 and 4.

Orally administered ^{14}C-labelled sulfoxaflor was rapidly absorbed without any apparent lag time, and C_{max} was reached within 1.6 hours or 2.3 hours for the low- or high-dose group, respectively (Table 3). Elimination of the radioactivity from plasma was rapid during the α elimination phase ($t_{½\alpha}$ = 4–6 hours), followed by relatively slow elimination during the terminal β phase ($t_{½\beta}$ = 39–45 hours). The AUC and C_{max} of radioactivity in plasma were essentially dose proportional between the low- and high-dose groups, suggesting unsaturated kinetic behaviour of sulfoxaflor up to 100 mg/kg bw. The kinetics (absorption, elimination half-life, AUC, clearance) of radioactivity in red blood cells were similar to those in plasma, except that detectable levels of radioactivity were found in red blood cells for an extended period of time when compared with plasma.

The per cent absorption of the orally administered dose in all three oral groups (single 5 mg/kg bw, multiple 5 mg/kg bw or single 100 mg/kg bw) was at least 92–96%, based on recovery in urine, non-gastrointestinal tissues and expired air (Table 4). Total recovery of radioactivity from all the animals averaged 102% and 108% in the oral and intravenous dose groups, respectively. The orally absorbed dose was rapidly excreted in urine (92–97%) without any sex difference. The majority of the urinary elimination (89–94%) occurred within the first 24 hours post-dosing. Only a small percentage (5–8%) of the oral dose was eliminated in faeces. The intravenously administered test material was also rapidly excreted in urine (97–101%). The majority of the urinary elimination (91–95%) occurred in the first 24 hours post-dosing. Only a small percentage (6–9%) of the intravenous dose was eliminated in faeces. Systemic bioavailability, calculated from the dose-corrected plasma AUC data for the low oral and intravenous dose groups, was 94% for both male and female rats.

Only 0.2–1.2% of the administered ^{14}C-labelled sulfoxaflor (oral administration: low single, high single or multiple dose) remained in the tissues after 168 hours (7 days) post-dosing. An average of 0.6–1.3% of the intravenous dose of ^{14}C-labelled sulfoxaflor remained in the tissues of the animals 168 hours post-dosing (Table 4).

The concentrations of radioactivity in tissues at termination were below 0.02 µg equivalent (eq) per gram for the low single oral dose group and for the intravenous dose group (except for skin,

Table 4. Recovery of radioactivity in tissues and excreta in rats 168 hours post-dosing with ^{14}C-labelled sulfoxaflor

	Recovery of radioactivity (% of administered dose)							
	5 mg/kg bw		100 mg/kg bw		5 mg/kg bw		5 mg/kg bw	
	Oral/single dose		Oral/single dose		Oral/multiple dose		Intravenous/single dose	
	Males	Females	Males	Females	Males	Females	Males	Females
Tissues	0.18	0.19	1.17	0.19	0.94	0.30	1.29	0.57
Final cage wash	0.44	0.62	1.20	0.91	9.92	0.42	0.21	0.39
Faeces	6.73	6.46	7.98	5.23	6.75	7.11	8.80	6.13
Urine/rinse	92.37	91.75	93.83	93.75	96.82	94.56	97.07	100.62
Expired volatiles, carbon dioxide	0	0	ND	ND	ND	ND	ND	ND
Total	100.2	99.7	104.4	100.4	103.4	102.4	107.7	108.0

From Hansen et al. (2009)
ND, not determined

0.08–0.19 µg eq/g). In the high single oral dose group, the highest concentrations were in skin (4.6 µg eq/g), red blood cells (0.57 µg eq/g), kidneys (0.36 µg eq/g), gastrointestinal tract (0.29 µg eq/g), liver (0.27 µg eq/g) and bladder (0.25 µg eq/g). The tissue concentrations of radioactivity from the multiple-dosed animals followed a similar trend to that observed in animals given a single low dose. Except for skin (0.05–0.18 µg eq/g), concentrations in all tissues for both male and female rats were below 0.03 µg eq/g (Table 5).

In summary, ^{14}C-labelled sulfoxaflor administered orally was rapidly and extensively absorbed, widely distributed throughout the body and readily eliminated, mainly via the urine, with low tissue residues (Hansen et al., 2009).

In a study on tissue distribution and metabolism of sulfoxaflor conducted according to OECD test guideline 417, groups of eight male and eight female F344/DuCrl rats received sulfoxaflor-2-^{14}C-pyridine (purity 95.6%; radiochemical purity 99.3%) as a 0.5% methylcellulose suspension by oral gavage at a single dose of 5 or 100 mg/kg bw; the dose volume was 5 ml/kg bw. Four rats of each sex per dose level were killed at predetermined times of either C_{max} plasma radioactivity (i.e. 1 and 0.5 hour post-dosing for males and females, respectively, at 5 mg/kg bw; 2 hours post-dosing for both males and females at 100 mg/kg bw) or ½ C_{max} plasma radioactivity (i.e. 7 and 6 hours post-dosing for males and females, respectively, at 5 mg/kg bw; 8 and 7 hours post-dosing for males and females, respectively, at 100 mg/kg bw). Urine, faeces, blood and tissues were collected and analysed for radioactivity. Additionally, the profile of sulfoxaflor-derived metabolites was investigated in pooled liver and kidney homogenates and pooled plasma samples.

Orally administered sulfoxaflor was rapidly absorbed without any apparent lag time. Total recovery of radioactivity in animals terminated at ½ C_{max} was greater than 98% for both dose levels (Table 6).

Test material–derived radioactivity (as micrograms ^{14}C-labelled sulfoxaflor equivalent per gram) was highest in the gastrointestinal tract, liver, kidney and urinary bladder, consistent with portal of entry and primary tissues of excretion. Plasma levels were approximately 50–60% of those found in the kidney and liver in both groups (Table 7).

In summary, administered sulfoxaflor was rapidly absorbed and widely distributed throughout the body, with the highest levels in portal of entry and excretory tissues. Test material–derived radioactivity in tissues (other than portal of entry and excretory tissues) tracks that of systemic blood, showing no potential for bioaccumulation (Rick et al., 2010).

Table 5. Recovery of radioactivity in tissues of rats 168 hours post-dosing with ^{14}C-labelled sulfoxaflor

	Recovery of radioactivity (µg eq/g)							
	5 mg/kg bw		100 mg/kg bw		5 mg/kg bw		5 mg/kg bw	
	Oral/single dose		Oral/single dose		Oral/multiple dose		Intravenous/single dose	
	Males	Females	Males	Females	Males	Females	Males	Females
Adrenals	0.011	0.009	0.235	0.195	0.012	NQ	0.011	0.009
Bladder	0.006	0.010	0.254	0.138	0.011	0.007	0.010	NQ
Blood	0.009	0.008	0.206	0.200	0.018	0.013	0.010	0.008
Bone	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
Bone marrow	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
Brain	0.007	0.005	0.199	0.125	0.007	NQ	0.007	0.006
Carcass	0.007	0.007	0.163	0.145	0.011	0.008	0.007	0.005
Fat	NQ	0.013	0.170	0.194	0.012	0.010	0.008	0.007
Gastrointestinal tract	0.010	0.015	0.184	0.292	0.011	0.006	0.013	0.006
Heart	0.007	0.008	0.185	0.167	0.006	0.007	0.008	0.006
Kidneys	0.012	0.014	0.303	0.358	0.017	0.012	0.014	0.014
Liver	0.010	0.009	0.269	0.209	0.018	0.011	0.013	0.008
Lungs	0.007	0.012	0.174	0.170	0.011	0.010	0.008	0.007
Lymph nodes	NQ	0.006	0.141	0.139	0.009	NQ	0.006	0.005
Muscle	0.006	0.006	0.149	0.124	0.009	0.006	0.007	0.005
Pancreas	0.007	0.008	0.172	0.161	0.010	0.007	NQ	0.005
Pituitary	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
Plasma	0.005	0.004	0.182	0.139	0.009	0.008	0.005	NQ
Red blood cells	0.009	0.009	0.570	0.327	0.024	0.022	0.014	0.013
Skin	0.010	0.011	4.608	0.304	0.183	0.049	0.187	0.080
Spleen	0.007	0.007	0.149	0.149	0.010	0.007	0.007	0.008
Testes/ovaries	0.007	0.006	0.165	0.116	0.008	0.006	0.007	0.005
Thymus	0.005	0.005	0.145	0.112	0.012	0.005	0.004	0.003
Thyroid	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
Uterus	—	0.008	—	0.118	—	0.006	—	0.005

From Hansen et al. (2009)
NQ, not quantifiable

1.2 Biotransformation

In the study on the toxicokinetics and metabolism of sulfoxaflor-2-^{14}C-pyridine in F344/DuCrl rats described above (Hansen et al., 2009), excreta from the animals were analysed for metabolites of sulfoxaflor.

Seven radiolabelled components (peaks A–H) were identified in urine and/or faecal samples. Parent sulfoxaflor was the primary component in both urine and faecal samples, accounting for a total of more than 93% of the administered dose.

In urine samples, six radioactive peaks (peaks B, C, D, E, F and G) were detected. Two of these peaks (peaks F and G) were consistently greater than 5% of the administered dose and were

Table 6. Recovery of radioactivity in tissues and excreta of rats at different time points after oral administration of ^{14}C-labelled sulfoxaflor

	Recovery of radioactivity (% of administered dose)							
	5 mg/kg bw				100 mg/kg bw			
	Males	Females	Males	Females	Males	Females	Males	Females
	Termination (h post-dosing)							
	1	0.5	7	6	2	2	8	7
Blood	2.81	2.55	1.60	1.55	2.04	2.06	1.22	1.11
Kidneys	1.07	0.96	0.65	0.69	0.77	0.78	0.57	0.49
Liver	6.58	5.74	3.57	3.39	4.07	3.39	2.88	2.51
Tissues, total	91.67	91.78	52.87	57.06	86.06	81.74	49.30	42.12
Final cage wash	5.32	2.98	16.36	10.77	11.01	17.07	11.19	17.98
Faeces	0	0	0.21	ND	0	0	0.11	0.05
Urine	0	0	29.04	30.23	0	0	41.00	40.25
Total	97.0	94.8	98.5	98.1	97.1	98.8	101.6	100.4

From Rick et al. (2010)
ND, not determined

Table 7. Recovery of radioactivity in tissues of rats at different time points after oral administration of ^{14}C-labelled sulfoxaflor

	Recovery of radioactivity (µg eq/g)							
	5 mg/kg bw				100 mg/kg bw			
	Males	Females	Males	Females	Males	Females	Males	Females
	Termination (h post-dosing)							
	1	0.5	7	6	2	2	8	7
Adrenals	8.09	8.73	4.50	6.10	130.3	161.23	66.00	96.25
Bladder	11.47	6.25	13.44	5.37	280.9	272.0	438.0	165.2
Blood	4.28	5.13	2.42	3.51	66.85	84.72	40.59	51.72
Bone	2.65	1.73	1.11	1.31	29.93	35.35	28.01	35.28
Bone marrow	4.77	5.25	2.65	3.71	73.42	87.00	49.44	57.95
Brain	2.98	2.84	1.69	2.46	37.39	44.63	27.37	35.22
Carcass	4.45	4.12	2.43	3.44	68.71	80.19	45.08	48.11
Fat	2.29	2.24	1.46	1.62	31.07	40.21	24.97	26.01
Gastrointestinal tract	12.31	24.30	5.05	8.11	380.1	481.4	141.0	146.6
Heart	5.13	6.62	3.20	4.69	90.47	111.5	56.92	68.77
Kidneys	8.04	8.34	4.70	6.10	109.2	136.2	83.17	89.99
Liver	10.93	11.94	5.68	7.77	129.9	143.7	82.40	101.1
Lungs	5.59	6.30	3.08	4.43	87.17	107.2	53.61	63.95
Lymph nodes	4.33	5.36	2.53	3.80	67.33	87.37	40.68	45.72
Muscle	5.00	5.16	2.93	4.07	77.10	97.84	49.35	58.77
Pancreas	6.47	7.59	3.72	5.46	101.2	124.9	63.69	81.58
Pituitary	5.57	6.45	3.35	4.82	84.80	107.3	57.70	66.25
Plasma	4.92	5.62	2.65	3.90	72.02	93.23	46.81	57.65
Red blood cells	3.95	4.95	2.32	3.45	63.54	82.50	41.49	49.56
Skin	3.62	3.62	2.15	2.72	49.95	63.32	25.66	38.97
Spleen	5.03	5.82	2.89	4.14	78.00	95.45	54.44	61.82
Testes/ovaries	4.07	5.86	2.93	4.27	65.15	100.43	48.85	61.38
Thymus	4.38	5.42	2.78	3.88	74.52	92.40	46.08	56.77
Thyroid	5.92	6.75	9.33	5.08	95.27	121.4	60.05	74.19
Uterus	—	5.35	—	3.94	—	92.71	—	55.18

From Rick et al. (2010)

identified as diastereomers of parent sulfoxaflor. Peak F accounted for 48.0–54.4% and peak G for 33.7–40.5% of the administered dose in all groups. Peak B (a glucuronide conjugate of the metabolite X11721061) accounted for 2.66–4.07% of the administered dose in all groups. Each of the remaining three metabolites (peaks C, D and E) accounted for less than 1% of the administered dose and was found only in pooled urine samples (Table 8).

In faecal samples, four metabolite peaks (peaks A, F, G and H) were consistently detected in all dose groups; none of these radioactive peaks was greater than 5% of the administered dose. Peaks F and G were identified as diastereomers of parent sulfoxaflor. Two minor components (peaks A and H), both less than 1% of the administered dose, were present only in extracts of faecal samples (Hansen et al., 2009).

In the study of the tissue distribution and metabolism of sulfoxaflor-2-^{14}C-pyridine in F344/DuCrl rats described above (Rick et al., 2010), pooled liver and kidney homogenates and pooled plasma samples were analysed for metabolites of sulfoxaflor.

Parent sulfoxaflor, identified as its two diastereomers, was the only component detected in the kidney, liver and plasma samples above the limits of detection of 0.286, 0.563 and 0.146 µg eq/g tissue, respectively, indicating that the metabolism of sulfoxaflor was minimal, at most (Rick et al., 2010).

The proposed metabolic scheme for sulfoxaflor in rats is illustrated in Figure 1.

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

Results of studies of the acute toxicity of sulfoxaflor are summarized in Table 9.

In an acute oral toxicity study conducted according to OECD test guideline 425 (2001, "up and down procedure"), male and female F344/DuCrl rats received sulfoxaflor (purity 95.6%) in 0.5% aqueous methylcellulose at a single dose of 630, 1000, 1580 or 2000 mg/kg bw by gavage in a volume of 10 ml/kg bw. Single animals were treated sequentially. Depending on the survival or death of the treated animal, the dose level administered to the next animal was increased or decreased, respectively. The rats were monitored daily for mortality and clinical signs for a 2-week period, and body weights were recorded on days 1, 2, 8 and 15. On day 15, surviving animals were killed, necropsied and examined for gross pathological changes.

One of two males and one of three females dosed with 1000 mg/kg bw died; additional mortalities were observed at a dose level of 1580 mg/kg bw (1/1 female; 1/1 male survived) and 2000 mg/kg bw (1/1 male; females not treated). In one animal of each sex treated with 630 mg/kg bw, muscle tremors and decreased activity were observed on day 1; these resolved by day 2. One female had decreased faeces on days 2 and 3. Animals treated with 1000, 1580 or 2000 mg/kg bw exhibited muscle tremors, twitches and/or tonoclonic convulsions. Further clinical signs were noted in some animals at these dose levels. Time of peak effect was approximately 2 hours post-dosing for most animals.

The estimated acute oral median lethal dose (LD_{50}) in rats was 1405 mg/kg bw in males and 1000 mg/kg bw in females (Brooks et al., 2008).

Table 8. Radioactive peaks detected in urine and faeces of rats dosed with ^{14}C-labelled sulfoxaflor

	% of administered dose							
	5 mg/kg bw		100 mg/kg bw		5 mg/kg bw		5 mg/kg bw	
	Oral/single dose		Oral/single dose		Oral/multiple dose		Intravenous/single dose	
	Males	Females	Males	Females	Males	Females	Males	Females
Peak A, faeces only	0.935	0.723	0.812	0.701	0.896	0.736	0.982	0.511
Peak B, urine only	2.88	2.66	3.42	3.52	3.26	3.25	3.80	4.07
Peak C, urine only	0.417	0.266	0.950	0.154	0.248	0.280	0.340	0.280
Peak D, urine only	0.750	0.659	0.621	0.600	0.660	0.817	0.758	0.716
Peak E, urine only	0.349	0.403	0.782	0.585	NQ	0.416	0.491	0.648
Peak F, urine and faeces	54.1	54.3	55.9	54.4	56.8	55.6	57.9	58.2
Peak G, urine and faeces	37.9	37.6	38.1	38.1	39.7	38.8	41.2	42.0
Peak H, faeces only	0.700	0.569	0.466	0.430	0.452	0.568	0.477	0.320
Total	98.1	97.1	101	98.5	102	100	106	107

From Hansen et al. (2009)
NQ, non-quantifiable

Figure 1. Proposed metabolic scheme for sulfoxaflor (XDE-208) in rats

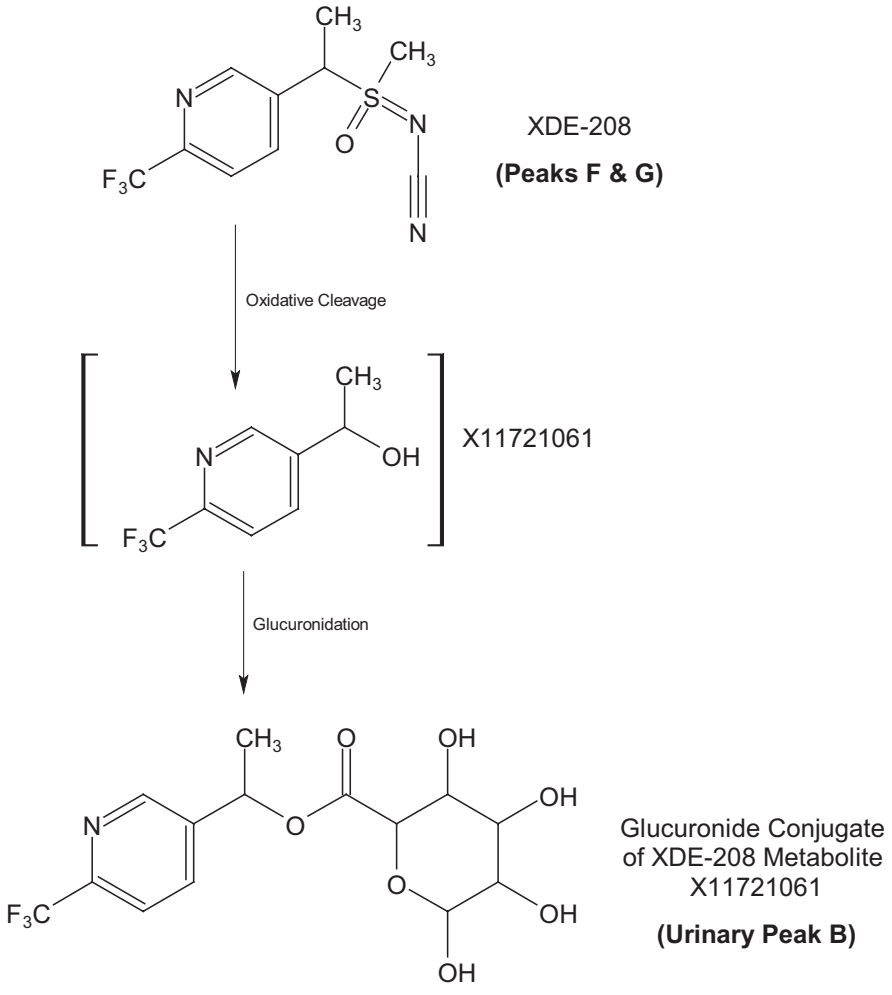


Table 9. Summary of acute oral, dermal and inhalation toxicity of sulfoxaflor

Species	Strain	Sex	Route	Purity (%)	LD_{50} (mg/kg bw) or LC_{50} (mg/l)	Reference
Rat	F344/DuCrl	M & F	Oral	95.6	M: 1405 F: 1000	Brooks et al. (2008)
Mouse	Crl:CD1(ICR)	M	Oral	95.6	750	Brooks, Wiescinski & Golden (2008)
Rat	Fischer 344	M & F	Dermal	95.6	> 5000	Durando (2008a)
Rat	F344/DuCrl	M & F	Inhalation	95.6	> 2.09	Krieger & Radtke (2009)

F, female; LC_{50}, median lethal concentration; LD_{50}, median lethal dose; M, male

In an acute oral toxicity study conducted according to OECD test guideline 425 (2001, "up and down procedure"), male (Crl:CD1(ICR)) mice received sulfoxaflor (purity 95.6%) in 0.5% aqueous methylcellulose at a single dose of 560, 750 or 1000 mg/kg bw by gavage in a volume of 10 ml/kg bw. Single animals were treated sequentially. Depending on the survival or death of the treated animal, the dose level administered to the next animal was increased or decreased, respectively. The animals were monitored daily for mortality and clinical signs for a 2-week period, and body weights were recorded on days 1, 2, 8 and 15. On day 15, surviving animals were killed, necropsied and examined for gross pathological changes.

One of three males dosed with 750 mg/kg bw died; additionally, the animal that received 1000 mg/kg bw died. The animal treated with 560 mg/kg bw showed laboured respiration, muscle convulsions, decreased activity and decreased resistance to removal on day 1, which resolved by day 2. Prior to death, one animal dosed at 750 mg/kg bw exhibited clinical signs that included muscle convulsions and increased activity. The surviving two animals exhibited decreased activity, muscle twitches, tremors and/or convulsions, decreased responsiveness to touch or increased reactivity to stimuli on test day 1, which resolved by test day 2. Prior to death, the animal dosed at 1000 mg/kg bw exhibited clinical signs that consisted of muscle twitches, tremors, convulsions, increased reactivity to stimuli and increased responsiveness to touch. The surviving animals in the 750 mg/kg bw group lost body weight on test day 2 and then gained weight on later measurements.

The estimated acute oral LD_{50} in male mice was 750 mg/kg bw (Brooks, Wiescinski & Golden, 2008).

In an acute dermal toxicity study conducted according to OECD test guideline 402 (1987), groups of five Fischer 344 rats of each sex were administered sulfoxaflor (purity 95.6%) at a single dose of 5000 mg/kg bw. The pure solid test substance was moistened with distilled water to achieve a dry paste by preparing a 50% (w/w) mixture, which was transferred to a gauze layer (5.1 cm × 7.6 cm). The gauze strip was placed on the rat's dorsal area and trunk and secured in place using adhesive tape. After 24 hours, the dressing was removed and the area cleansed with soap solution and tap water, followed by gentle drying of the area with a paper towel. The rats were observed for clinical signs and mortality for at least 14 days, and body weight was checked weekly. On day 15, surviving animals were terminated, necropsied and examined for gross pathological changes.

No clinical signs were observed, and no mortalities occurred at 5000 mg/kg bw, the only dose tested. No abnormalities were observed at gross necropsy. The dermal LD_{50} was greater than 5000 mg/kg bw for male and female rats (Durando, 2008a).

In an acute inhalation toxicity study conducted according to OECD test guideline 403 (1981), groups of five (F344/DuCrl) rats of each sex were exposed (nose only) to a dust aerosol of sulfoxaflor (purity 95.6%) at a concentration of 2.09 mg/l for 4 hours. The observation period lasted for 14 days.

On day 15, all animals were terminated, necropsied and examined for gross pathological changes. Measurements of particle size distribution showed a mass median aerodynamic diameter (MMAD) of 3.6 µm (geometric standard deviation [GSD] ± 1.33 µm); 12% of the total particulate mass had an aerodynamic diameter below 1.3 µm, and 96% of the particulate mass was present in size fractions with an aerodynamic diameter below 6.1 µm.

No mortality occurred at the tested concentration. All rats tolerated the exposure with some evidence of reversible clinical signs in two female rats (soiling of the haircoat during the exposure period, perineal soiling during the post-exposure period). Body weight loss was observed on test day 2, but by day 4, the pre-exposure body weights were exceeded. No abnormalities were observed at gross necropsy.

Under the conditions of the study, the median lethal concentration (LC_{50}) for male and female rats after dust inhalation was greater than 2.09 mg/l, which was the highest attainable concentration (Krieger & Radtke, 2009).

(b) Dermal and ocular irritation and dermal sensitization

Results of the studies on dermal and eye irritation and dermal sensitization are summarized in Table 10.

In a study of skin irritation potential conducted according to OECD test guideline 404 (2002), 0.5 g of sulfoxaflor (purity 95.6%) moistened with distilled water was applied to the shorn dorsal skin of three female New Zealand White rabbits under a semi-occluded dressing for 4 hours. Skin reactions were scored at 1, 24, 48 and 72 hours post-treatment.

No signs of systemic toxicity or mortality were observed during the study period. Very slight erythema was seen in all animals 1 and 24 hours after the treatment and in one animal 48 hours after the treatment. Very slight oedema was observed in all animals 1 hour after the treatment. Seventy-two hours after removal of the patch, scores for erythema and oedema were 0 for all three animals. It was concluded that sulfoxaflor is non-irritating to rabbit skin according to the European Union (EU) classification criteria (Classification, Labelling and Packaging Regulation, Dangerous Substances Directive) (Durando, 2008b).

In a study of eye irritation potential conducted according to OECD test guideline 405 (2002), 0.1 ml of sulfoxaflor (purity 95.6%) was placed into the conjunctival sac of one eye of each of three male New Zealand White rabbits. Ocular lesions were scored at 1, 24, 48 and 72 hours post-instillation.

No signs of systemic toxicity were observed during the study period. No signs of corneal opacity were observed in any of the test animals during the study. One hour after test substance instillation, all three treated eyes exhibited positive conjunctivitis, and iritis was evident in two eyes. The overall incidence and severity of irritation decreased with time. All animals were free of ocular irritation within 72 hours. The individual averages of the scores for the 24-, 48- and 72-hour observation periods for conjunctival redness (which was the most responsive parameter) were 0.3, 1 and 1 for all three test animals. It was concluded that sulfoxaflor is not irritating to the eyes according to the EU classification criteria (Classification, Labelling and Packaging Regulation, Dangerous Substances Directive) (Durando, 2008c).

In a study of skin sensitization potential conducted according to OECD test guideline 429 (2002, local lymph node assay), sulfoxaflor (purity 95.6%) was topically applied to the dorsal surface of each ear of female CBA/J mice. Groups of six mice per dose were dosed once daily for 3 consecutive days with 25 µl of a formulation of the test compound at concentrations of 5%, 25% and 50% in dimethylsulfoxide (DMSO). These dose levels were chosen on the basis of preliminary

Table 10. Summary of irritation and skin sensitization potential of sulfoxaflor

Species	Strain	Sex	End-point	Purity (%)	Result	Reference
Rabbit	New Zealand White	F	Skin irritation	95.6	Not irritating	Durando (2008b)
Rabbit	New Zealand White	M	Eye irritation	95.6	Not irritating	Durando (2008c)
Mouse	CBA/J	F	Skin sensitization (LLNA)	95.6	Not sensitizing	Wiescinski & Sosinski (2008)

F, female; LLNA, local lymph node assay; M, male

results showing that concentrations of 1%, 5%, 10%, 20%, 40% and 50% caused no effects on body weight, and erythema was not induced. Two additional groups received the vehicle (DMSO) or α-hexylcinnamaldehyde (30%) to serve as negative and positive controls, respectively. Animals were checked for mortality and clinical signs at least daily during the study. In particular, the site of application was examined for signs of local irritation. Individual body weights were measured at study start and at scheduled termination.

No mortality or clinical signs were observed during the study. In particular, no cutaneous reactions were observed at the application site of animals treated with the test compound. Body weight changes were comparable between control and treated groups. Negative responses were observed at all tested dose levels (stimulation indices: 1.0, 1.1 and 1.0 for 5%, 25% and 50% concentrations, respectively). The results of the positive control demonstrated the validity of the assay (stimulation index: 12.0). On the basis of this study, sulfoxaflor did not show any sensitization potential and does not warrant classification or labelling as a skin sensitizer (Wiescinski & Sosinski, 2008).

2.2 Short-term studies of toxicity

Mice

In a palatability study, groups of 4–5 female Crl:CD1(ICR) mice were fed diets containing sulfoxaflor (purity 98.3%) at concentrations of 0, 2000, 3000, 4500 or 6000 parts per million (ppm), equal to 0, 317, 418, 345 and 462 mg/kg bw per day, for 3–7 days. Initially, groups of five female mice were given a diet containing 0 or 2000 ppm daily for 7 days. No significant effects were noted, and the mice were necropsied on study day 8 along with a group of three control mice, which were initially excluded from the study following randomization. The original five control mice remained on study for comparison with subsequent treatment groups. As no significant effects were observed in mice given 2000 ppm, an additional group of five female mice received diets containing 3000 ppm daily for up to 7 days. No significant effects were noted; therefore, two additional groups of four female mice received diets containing 4500 or 6000 ppm and were terminated after 3 days on study because of decreases in feed consumption and body weights. The five female mice in the control group given untreated feed throughout the study were necropsied with the mice in the 4500 and 6000 ppm dose groups. Animals were evaluated by daily body weights, feed consumption and cage-side examinations. A complete necropsy was conducted on all animals, and liver weights were recorded at necropsy. Samples of liver tissue from animals in the control, 4500 ppm and 6000 ppm groups were collected after 3 days of treatment for possible future enzyme analyses. Blood samples were collected from all animals (treated and controls) at necropsy and stored at −80 ºC for possible toxicokinetic analysis. Histological evaluation of the liver from all animals was conducted.

The 4500 and 6000 ppm dose groups were terminated after 3 days of treatment for humane reasons based on decreased feed consumption (reduced to 46% and 41% of control, respectively), excessive feed scratching and body weight decrements (10% and 16%, respectively, compared with day 1 values). The poor palatability of sulfoxaflor in the diet resulted in decreased feed consumption

and a lower test material intake value at 4500 ppm (345 mg/kg bw per day) than at 3000 ppm (418 mg/kg bw per day). Absolute liver weights were increased by 34% and 27% and relative liver weights were increased by 40% and 44%, relative to concurrent controls, for mice given 4500 and 6000 ppm, respectively. These liver weight increases were considered related to treatment.

Mice given 3000 ppm were observed with scratched feed (four of five animals) on test day 2, and the available feed consumption value was decreased by 39% compared with controls. Mean body weights for these animals were decreased by approximately 4% relative to controls, and one individual body weight had decreased by 7% when compared with its value on day 1. However, at termination, feed consumption and body weight values were comparable to those of controls. Liver weights (absolute and relative) of animals given 3000 ppm were increased by 40% and 53.4%, respectively, when compared with the nearest available historical control averages (values for concurrent controls were not available during this study phase), and hence the increases were interpreted to be treatment related.

Animals given 2000 ppm had decreases in mean body weights and feed consumption of 5% and 31%, respectively, on study day 2 when compared with controls. These animals quickly adjusted to the feed, and, by study day 4, body weights and feed consumption values were comparable to those of controls. At necropsy (day 8), absolute and relative liver weights were increased by 17% and 21%, respectively, relative to concurrent controls, and the increases were considered to be treatment related.

Treatment-related histopathological changes in the liver consisted of hepatocellular hypertrophy (with altered tinctorial properties) in the majority of animals given 2000 ppm and above. A very slight overall increase in the numbers of mitotic figures was present in the majority of the livers from animals given 2000 or 3000 ppm and from some given 4500 or 6000 ppm, compared with the controls, indicating increased hepatocyte proliferation. Occasional, scattered, individual necrotic hepatocytes were observed in some animals given 4500 or 6000 ppm. Some animals given 4500 or 6000 ppm had very slight or slight hepatic fatty change consistent with reduced feed consumption (Table 11).

Under the conditions of this study, the highest palatable dietary concentration of sulfoxaflor for female CD-1 mice was 3000 ppm. At concentrations of 4500 ppm and above, sulfoxaflor was not sufficiently palatable to maintain acceptable growth and to be used in subsequent studies (Thomas & Dryzga, 2010).

In a study of toxicity conducted according to OECD test guideline 407, groups of five male and five female CD-1 mice were fed diets containing sulfoxaflor (purity 98.3%) at a concentration of 0, 300, 1500 or 3500 ppm, equal to 0, 43.9, 230 and 524 mg/kg bw per day for males and 0, 53, 273 and 638 mg/kg bw per day for females, for 28 days. Satellite groups of three male and three female CD-1 mice were fed diets supplying 0 or 1500 ppm sulfoxaflor for 3 days (termination on day 4). The livers of mice in the satellite group were collected and stored for possible future mechanistic studies. Parameters evaluated were daily cage-side observations, weekly detailed clinical observations, ophthalmic examinations, body weights, feed consumption, haematology, clinical chemistry, selected organ weights, and gross pathological and histopathological examinations.

Feed consumption for males and females given 1500 or 3500 ppm was lower than for the controls during days 1–2, whereas feed consumption was comparable to that of the controls after day 4. Slight treatment-related decreases in body weights and body weight gains were observed in males and females given 1500 or 3500 ppm during the 1st week of the study. Body weight gains of males given 3500 ppm were only 50% of the control values on day 8, but subsequently improved and were comparable to those of controls throughout the rest of the study. There was a loss of body weight in high-dose females through day 4. However, body weight and body weight gains were comparable to those of controls from day 5 through the rest of the study. Body weight gains of males and females

Table 11. Summary of selected histopathological findings in hepatocytes of mice fed diets containing sulfoxaflor for 3–7 days

	Incidence of finding				
	Dietary concentration (ppm)				
	0	2000	3000	4500	6000
Number of mice examined	*8*	*5*	*5*	*4*	*4*
Altered tinctorial properties					
- very slight	0	1	0	1	0
Hypertrophy, with altered tinctorial properties					
- very slight	0	0	0	3	2
- slight	0	4	5	0	2
Mitotic alteration, increased, multifocal					
- very slight	0	3	5	2	1
Necrosis, individual cells, multifocal					
- very slight	0	0	0	2	3
Vacuolation, consistent with fatty change					
- very slight	0	0	0	2	2
- slight	0	0	0	2	1

From Thomas & Dryzga (2010)

given 1500 ppm were comparable to those of controls from day 4 and day 3 through the rest of the study, respectively.

At termination on day 30, males and females given 1500 or 3500 ppm had treatment-related elevations in mean serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities (only changes in ALT were statistically significant). Triglyceride levels and serum alkaline phosphatase (ALP) activity were also elevated in males and females given 3500 ppm (Table 12).

At necropsy, there were treatment-related increases in the mean absolute and relative liver weights of males and females given 1500 ppm (28% and 30% in males and 16% and 19% in females, respectively) or 3500 ppm (93% and 93% in males and 44% and 48% in females, respectively) compared with controls (Table 12). Increases in liver weights were associated with treatment-related centrilobular/midzonal hepatocyte hypertrophy in the livers of males and females given 1500 or 3500 ppm. Males given 300 ppm had marginal treatment-related increases in mean absolute (15%) and relative (11%) liver weights over the respective control values, but these were not statistically significant and were not associated with detectable hepatocyte hypertrophy or clinical chemistry changes and were therefore considered a non-adverse effect. Other treatment-related histopathological changes consisted of a very slight or slight increase in hepatocyte mitoses and very slight hepatocyte fatty change in the livers of males given 1500 or 3500 ppm. Treatment-related multifocal necrosis of scattered individual hepatocytes was present in the livers of males given 1500 or 3500 ppm and in females given 3500 ppm. Males given 3500 ppm had elevations in absolute and relative adrenal gland weights. Although these were not statistically significant, they exceeded the historical control range and were associated with hypertrophy of the zona fasciculata of the adrenal cortex. They were therefore considered to be treatment related. Males given 3500 ppm also had very slight or slight treatment-related atrophy of the mesenteric adipose tissue.

Toxicokinetic analysis of the plasma from the above animals showed that dose-proportional intake of sulfoxaflor was translated into a dose-proportional increase in plasma concentrations of sulfoxaflor (Table 12). The systemic exposure of sulfoxaflor was approximately 40% higher in males

Table 12. Summary of selected findings in mice fed diets containing sulfoxaflor for 28 days

	Males				Females			
	Dietary concentration (ppm)							
	0	300	1500	3500	0	300	1500	3500
Plasma concentration (µg/g), day 26 or 30	0	23.7[a]	49.3	104	0	7.11	35.32	73.3
ALT (U/l)	38	55	258*	193*	37	45	68*	77*
ALP (U/l)	80	60	96	127*	87	105	106	128*
AST (U/l)	82	79	186	148	87	90	126	109
Triglycerides (mg/dl)	95	98	110	221*	74	62	89	123*
Calcium (mg/dl)	9.6	9.7	10.0	10.1*	10.1	10.2	10.4	10.4*
Liver weight, absolute (g)	1.73	1.98	2.20	3.33*	1.37	1.35	1.59	1.97*
Liver weight, relative (% of body weight)	4.87	5.42	6.32*	9.42*	4.90	4.92	5.84*	7.26*
Kidney weight, absolute (g)	0.63	0.67	0.54	0.54*	0.39	0.35	0.35	0.35*
Kidney weight, relative (% of body weight)	1.77	1.83	1.57	1.53*	1.41	1.29	1.27	1.30*
Hepatocytes (no.)								
- hypertrophy	0	0	5	5	0	0	5	5
- mitotic alteration	0	0	1	4	0	0	0	0
- single-cell necrosis, focal	1	1	0	0	1	0	0	0
- single-cell necrosis, multifocal	0	0	4	5	0	0	0	4
- vacuolation/fatty change	0	0	1	4	0	0	0	0
Adrenals, hypertrophy zona fasciculata (no.)	0	0	0	5	0	0	0	0

From Thomas et al. (2008)
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; U, units; * $P < 0.05$
[a] Mean = 10.48 after removing one outlier.

than in females. The urinary elimination of sulfoxaflor in males was between 33% and 44% and in females was between 23% and 65% of what they consumed during a 24-hour period. Only parent sulfoxaflor was observed in the chromatograms of the plasma samples from the sulfoxaflor-dosed mice. In addition to parent sulfoxaflor, urine samples contained one potential metabolite, which was present at less than 2% of the parent sulfoxaflor concentration, based on equimolar mass spectroscopic response, indicating that the majority of the dietary administered sulfoxaflor was excreted essentially unchanged in the urine.

The no-observed-adverse-effect level (NOAEL) was 300 ppm (equal to 43.9 mg/kg bw per day), based on liver toxicity (increased serum ALT, multifocal single-cell necrosis and vacuolation/fatty change of hepatocytes) in males at 1500 ppm (equal to 230 mg/kg bw per day) and above (Thomas et al., 2008).

In a study of toxicity conducted according to OECD test guideline 408, groups of 10 male and 10 female CD-1 mice were fed diets containing sulfoxaflor (purity 96.6%) at a concentration of 0, 100, 750 or 1250 ppm for males and at a concentration of 0, 100, 1500 or 3000 ppm for females, for at least 90 days. These concentrations corresponded to time-weighted average doses of 0, 12.8, 98.0 and 166 mg/kg bw per day for males and 0, 16.2, 247 and 489 mg/kg bw per day for females. Parameters evaluated were daily cage-side observations, weekly detailed clinical observations, ophthalmic examinations, body weights, feed consumption, haematology, clinical chemistry, selected organ weights, and gross pathological and histopathological examinations. In addition, toxicokinetic analyses were conducted on urine (day 80) and terminal blood plasma.

There were no treatment-related effects on clinical signs, ophthalmic parameters, body weights or feed consumption.

There was a minor reduction in haematocrit and haemoglobin concentration in females given 1500 or 3000 ppm. Males given 1250 ppm had statistically significant increases in the activities of serum ALT by 200%, AST by 43% and ALP by 142%. Serum total cholesterol and total bilirubin concentrations were statistically significantly decreased in males given 750 and 1250 ppm, to below the respective historical control range. Females given 1500 or 3000 ppm had elevations in the activities of serum ALT (125% and 171% increase, respectively) and AST (44% and 31% increase, respectively) and decreased ALP activity (3000 ppm only) compared with respective controls, but these only occasionally reached statistical significance, although for AST and ALP, values were outside the respective historical control range. Serum triglycerides were elevated in females given 1500 or 3000 ppm, and serum cholesterol concentration was elevated in females given 3000 ppm (Table 13).

Males given 750 or 1250 ppm and females given 1500 or 3000 ppm had treatment-related increases in absolute (20% and 74% in males and 36% and 54% in females, respectively) and relative liver weights (26% and 85% in males and 40% and 50% in females, respectively) compared with the respective controls. Other treatment-related organ weight changes consisted of increased absolute and relative adrenal weights in males given 1250 ppm and lower absolute kidney weights in males given 750 or 1250 ppm. The lower kidney weights were, however, considered non-adverse because of the lack of any treatment-related histopathological changes in the kidneys of the high-dose males. Absolute and relative thymus weights were decreased in high-dose females.

Males given 750 or 1250 ppm and females given 1500 or 3000 ppm had slight or moderate, treatment-related, centrilobular to midzonal hepatocyte hypertrophy, with altered tinctorial properties (increased cytoplasmic eosinophilia). Other treatment-related histological liver effects consisted of an overall, very slight or slight increase in the numbers of mitotic figures (hepatocytes in mitosis) in the liver of males given 1250 ppm and very slight fatty change in hepatocytes of males given 750 or 1250 ppm. Males given 750 or 1250 ppm had treatment-related, very slight necrosis of scattered, individual hepatocytes, whereas this change in females given 1500 or 3000 ppm was infrequent or minimal (Table 13). Males given 750 or 1250 ppm and females given 1500 or 3000 ppm had treatment-related, very slight hypertrophy of the zona fasciculata of the adrenal cortex. A very slight, treatment-related fatty change was also present in the zona fasciculata of the adrenal cortex in some females given 1500 or 3000 ppm. Four out of 10 females given 3000 ppm had a very slight, treatment-related increase in extramedullary erythrocytic haematopoiesis in the spleen.

Toxicokinetic analysis of the plasma (based on the daily test material intake during the last 7 days of the study) showed that dose-proportional intake of sulfoxaflor translated into a dose-proportional increase in plasma concentrations of sulfoxaflor only up to the middle dose for both male (750 ppm, 92.3 mg/kg bw per day) and female (1500 ppm, 227 mg/kg bw per day) mice. In males, the systemic exposure, as measured by the plasma concentration of sulfoxaflor, became supra-linear between the middle (92.3 mg/kg bw per day) and high (152 mg/kg bw per day) doses (3.9-fold increase instead of 1.6-fold expected from the difference in the test material intake between the middle and high doses). Conversely, in females, plasma concentrations of sulfoxaflor reached a plateau, remaining almost unchanged between the middle (227 mg/kg bw per day) and the high (467 mg/kg bw per day) doses (Table 13). Total elimination of sulfoxaflor in 24-hour urine remained dose proportional only up to the middle dose and showed less than a dose-proportional increase at the highest dose, in both male and female mice. These data are consistent with a saturation of elimination of sulfoxaflor in male mice at the highest dose and a saturation of absorption of sulfoxaflor from the gastrointestinal tract in female mice at the highest dose. On the basis of these results, the kinetically derived maximum dose (i.e. the dose above which kinetics become non-linear) was considered to be 92.3 and 227 mg/kg bw per day (750 and 1500 ppm) for male and female mice, respectively.

Table 13. Summary of selected findings in mice fed diets containing sulfoxaflor for 90 days

	Males				Females			
	Dietary concentration (ppm)							
	0	100	750	1250	0	100	1500	3000
Substance intake (mg/kg bw per day), week 13	0	10.7	92.3	152	0	14.7	227	467
Plasma concentration (µg/g), week 13	0	2.1	16.8	65.7	0	1.4	18.1	17.9
Haemoglobin (g/dl)	15.0	14.8	15.1	14.5	15.7	15.3	14.9*	14.8*
Haematocrit (%)	49.4	48.8	49.2	48.3	50.9	49.7	48.7*	48.2*
ALT (U/l)	41	44	53	123*	28	33	63*	76
AST (U/l)	72	76	73	103*	97	104	140	127
ALP (U/l)	45	42	50	109*	83	85	72	61*
Cholesterol (mg/dl)	157	150	124*	89*	80	86	84	124*
Triglycerides (mg/dl)	101	77	106	127	70	72	105*	131*
Liver weight, absolute (g)	1.79	1.87	2.16*	3.13*	1.41	1.37	1.91*	2.17*
Liver weight, relative (% of body weight)	4.22	4.45	5.30*	7.73*	4.28	4.21	6.00*	6.43*
Adrenal weight, absolute (mg)	7.8	8.7	8.6	10.3	10.0	10.4	11.4	11.2
Adrenal weight, relative (% of body weight)	0.018	0.021	0.021	0.026*	0.031	0.032	0.036	0.034
Hepatocytes, hypertrophy (no.)								
- very slight	9	9	0	0	0	0	1	0
- slight	0	0	9	1	0	0	9	3
- moderate	0	0	1	9	0	0	0	7
Hepatocytes, mitotic alteration (no.)	0	0	0	10	0	1	0	0
Hepatocytes, necrosis, focal/multifocal (no.)	0	0	8	10	0	0	5	4
Hepatocytes, vacuolation/fatty change (no.)	0	0	6	10	0	0	0	1
Adrenals/zona fasciculata, hypertrophy (no.)	2	1	4	10	3	2	7	10
Adrenals/zona fasciculata, vacuolation/fatty change (no.)	0	0	0	0	0	0	5	2

From Thomas et al. (2010a)
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; U, units; * $P < 0.05$

The NOAEL was 100 ppm (equal to 12.8 mg/kg bw per day), based on effects in the liver (vacuolation/fatty change of hepatocytes) and the adrenals (hypertrophy and/or vacuolation of the zona fasciculata) observed in males at 750 ppm (equal to 98.0 mg/kg bw per day) and above and in females at 1500 ppm (equal to 247 mg/kg bw per day) and above (Thomas et al., 2010a).

Rats

In a palatability study, groups of five female F344/DuCrl rats were fed diets containing sulfoxaflor (purity 98.1%) at a concentration of 0, 2748, 5495 or 10 990 ppm (equal to 0, 198, 256 and 291 mg/kg bw per day) for up to 8 days to evaluate palatability and perform toxicokinetic analysis in blood plasma. Parameters evaluated were daily cage-side observations, body weights, feed consumption and gross examinations.

Feed consumption was reduced by 59%, 74% or 84% in animals given 2748, 5495 or 10 990 ppm, respectively, relative to controls following 1 day of administration. This was attributed to decreased palatability of sulfoxaflor in rodent feed. The reduction in feed consumption was associated with decreases in body weight at all dose levels. All animals fed 5495 or 10 990 ppm had marked body weight loss by days 5 and 4, respectively, and were humanely euthanized on that day. Animals

fed 2748 ppm lost up to 6.1 g body weight by day 4; however, there was a slight recovery in feed consumption (31% reduction) and body weights (18% reduction) by day 8, relative to controls. Sulfoxaflor was present in the plasma of rats fed 2748 ppm at a mean concentration of 72.8 µg eq/g plasma on day 8. No metabolites were detected in the plasma.

Therefore, under the conditions of this study, sulfoxaflor was not sufficiently palatable at concentrations greater than 2748 ppm (198 mg/kg bw per day) for these dose levels to be used in future dietary toxicity studies. Sulfoxaflor was quantifiable in rat plasma, and there were no metabolites detected (Yano et al., 2009a).

In a study of toxicity conducted according to OECD test guideline 407, groups of five male and five female F344/DuCrl rats were fed diets containing sulfoxaflor (purity 98.1%) at a concentration of 0, 300, 1000, 2000 or 3000 ppm (equal to 0, 24.8, 79.4, 155 and 205 mg/kg bw per day for males and 0, 26.5, 88.3, 170 and 192 mg/kg bw per day for females) for 28 days. Animals were observed daily for mortality and clinical signs. A detailed physical examination was performed weekly. Body weight and feed consumption were recorded frequently throughout the study. Blood samples for toxicokinetic analysis were taken on day 27 and for haematology and clinical chemistry determinations on day 29 (before final necropsy), whereas urine samples were collected during the last week of the study. At study termination, all animals were necropsied, selected organs were weighed and a range of tissues were taken, fixed and examined microscopically.

Administration of sulfoxaflor to male and female rats at 3000 ppm resulted in excessive reductions in feed consumption (by 31% and 36%) and marked body weight loss (by 21% and 19%) compared with controls after 9 days of administration, respectively; therefore, all animals in the 3000 ppm dose group were humanely euthanized (day 9). The lower feed consumption due to decreased palatability was responsible for the decreased body weights.

Feed consumption at 300, 1000 or 2000 ppm was decreased by 5%, 29% or 54% in males and by 7%, 26% or 48% in females, respectively, after 1 day of administration, but increased for the remainder of the study. By the end of the study, feed consumption for males given 300 or 1000 ppm was comparable to that of controls and was decreased by 6% at 2000 ppm. Females given 300, 1000 or 2000 ppm consumed 8%, 6% or 11% less than controls at the end of the study, respectively.

Animals given 1000 or 2000 ppm had decreased body weight after 1 day of exposure, and this was considered secondary to the reduced feed consumption. These animals gained weight for the remainder of the study. By day 28, males and females given 2000 ppm weighed 8.5% and 10% less, respectively, than controls. Males and females given 300 or 1000 ppm had body weights comparable to those of controls on day 28.

Males (all treated doses) and females (1000 or 2000 ppm) had dose-related increases in serum total cholesterol levels (Table 14). Males and females given 1000 or 2000 ppm also had total serum protein levels that were higher than those of controls. Albumin and globulin levels were marginally increased in males given 1000 or 2000 ppm and in females given 2000 ppm.

Males and females given 1000 or 2000 ppm had increased absolute and relative liver weights that were dose and treatment related. There were a number of additional differences in organ weights of males and females given 2000 ppm that were statistically significant, including relative brain (males and females; increased), relative kidney (males; increased), relative testes (males; increased), relative thyroid (males and females; increased), absolute heart (males; decreased) and absolute spleen (males and females; decreased). These differences in organ weights were considered to be secondary to the lower body weights of this dose group and did not reflect a primary target organ effect of sulfoxaflor. This conclusion is supported by the absence of histopathological changes in these organs.

Treatment-related histological effects were observed in the livers of males and females given 1000 or 2000 ppm and consisted of a dose-related increase in the severity (very slight to moderate)

Table 14. Summary of selected findings in rats fed diets containing sulfoxaflor for 28 days

	Males				Females			
	Dietary concentration (ppm)							
	0	300	1000	2000	0	300	1000	2000
Dose (mg/kg bw per day), day 27	0	20.2	67.3	137	0	22.2	74.8	151
Plasma concentration (µg/g), day 27	0	9.0	29.4	59.3	0	7.3	25.5	51.5
Plasma $AUC_{24\ h}$ (µg·h/ml), day 27	0	210	693	1371	0	167	591	1183
Plasma $t_{½}$ (h), day 27	0	7.88	8.30	7.19	0	4.46	5.46	4.92
Cholesterol (mg/dl)	51	74*[a]	110*	157*	74	82	104*	142*
Total protein (g/dl)	6.9	6.9	7.4[#]	7.6[#]	6.2	6.1	6.3[#]	6.6[#]
Albumin (g/dl)	4.1	4.0	4.3	4.3[#]	3.8	3.7	3.8	3.9[#]
Globulin (g/dl)	2.8	2.8	3.1	3.3	2.4	2.4	2.5	2.7
Body weight, terminal (g)	199.7	201.3	195.6	181.7*	134.4	125.7	129.3	119.3*
Liver weight, absolute (g)	6.46	6.50	8.14*	9.36*	4.20	3.82	4.34	5.05*
Liver weight, relative (% of body weight)	3.23	3.23	4.16*	5.15*	3.12	3.04	3.36	4.23*
Hepatocytes, hypertrophy (no.)								
- very slight	0	0	0	0	0	0	3	2
- slight	0	0	2	0	0	0	0	3
- moderate	0	0	3	5	0	0	0	0
Hepatocytes, vacuolation/fatty change (no.)								
- very slight	0	0	2	3	0	0	0	1
- slight	0	0	3	0	0	0	0	0

From Yano et al. (2009b)

AUC, area under the curve; $t_{½}$, half-life; * $P < 0.05$ (Dunnett's test, males and females analysed separately); [#] $P < 0.05$ (Dunnett's test, males and females analysed together)

[a] Outside historical control range (36–51 mg/dl) from five studies.

of hepatocellular hypertrophy (with altered tinctorial properties) involving the centrilobular to mid-zonal regions of the hepatic lobule (Table 14). Effects were more prominent in males than in females, increasing to moderate severity in 1000 and 2000 ppm males. Vacuolation, consistent with fatty change, involving hepatocytes primarily in the right lateral lobe was also occasionally seen in a multifocal distribution in males given 1000 or 2000 ppm and in one female given 2000 ppm. The restriction of this alteration to only one liver lobe, the minor nature of the effect (very slight or slight) and the lack of a clear dose–response relationship in regards to severity suggest that this was not a toxicologically significant effect.

Toxicokinetic analysis of the plasma showed that levels of sulfoxaflor ($AUC_{24\ h}$) were effectively proportional to dose, with a 3.3- to 3.6-fold increase between the 300 and 1000 ppm groups and a 2.0-fold increase between the 1000 and 2000 ppm dose groups. Females were more efficient than males in eliminating the test material. The 24-hour systemic dose as measured by the $AUC_{24\ h}$ was 21%, 15% and 14% lower in females than in males in the 300, 1000 and 2000 ppm nominal dose groups, respectively (corresponding to 210, 693 and 1371 µg·h/ml versus 167, 591 and 1183 µg·h/ml at the low, middle and high doses, respectively). The plasma elimination half-life of sulfoxaflor in male rats was between 7 and 8 hours, whereas it was 32–43% shorter in females (between 4 and 5 hours). The chromatograms of the plasma samples taken from sulfoxaflor-dosed rats contained up to five peaks in addition to the parent; however, these impurities and/or metabolites made up less than 1% of the total test material in the plasma.

The NOAEL was 300 ppm (equal to 24.8 mg/kg bw per day), based on marginal liver toxicity (increased serum cholesterol and total protein levels) in males at 1000 ppm (equal to 79.4 mg/kg bw per day) and above (Yano et al., 2009b).

In a study of toxicity conducted according to OECD test guideline 408, groups of 10 male and 10 female F344/DuCrl rats were fed diets containing sulfoxaflor (purity 96.6%) at a concentration of 0, 100, 750 or 1500 ppm (equal to 0, 6.36, 47.6 and 94.9 mg/kg bw per day for males and 0, 6.96, 51.6 and 101 mg/kg bw per day for females) for 90 days. An additional 10 males and 10 females fed either 0 or 1500 ppm of test diet for 90 days were maintained for 28 days after withdrawing the test diets to examine the reversibility of any effects seen. Parameters evaluated were daily cage-side observations, weekly detailed clinical observations, ophthalmic examinations, functional observational battery (FOB) (pre-exposure and prior to necropsy, comprising cage-side, hand-held and open-field observations, rectal temperature, forelimb and hindlimb grip performance, landing foot splay and motor activity), body weights, feed consumption, prothrombin time, haematology, urinalysis, clinical chemistry, selected organ weights and gross pathological and histopathological examinations, which included a specifically detailed review of the nervous system. The study also included integrated toxicokinetics and an assessment of immunotoxicity, as measured by immune responsiveness in the sheep red blood cell antibody-forming cell (AFC) assay.

There were no mortalities, no treatment-related clinical observations and no ophthalmic findings at any dose level. Also, there were no statistically significant or treatment-related FOB observations and no treatment-related effects on grip performance, landing foot splay or motor activity.

Feed consumption was dose-relatedly decreased in males (all treated doses) and females (750 and 1500 ppm), due to decreased palatability of diets containing sulfoxaflor. During the first 4 days, males given 100, 750 or 1500 ppm consumed 5%, 12% or 24% less feed than controls, and females given 750 or 1500 ppm consumed 8% or 21% less feed than controls. However, feed consumption for males at all dose levels was comparable to that of controls by the end of the study. Female feed consumption in the 750 and 1500 ppm groups was 5% or 8% lower than that of controls, respectively, at 90 days and statistically significantly different.

After 4 days of treatment, body weight gains of males given 750 or 1500 ppm were 24% or 45% lower than those of controls, respectively, and body weight gains of females given 750 or 1500 ppm were 13% or 60% lower than those of controls, respectively. These animals gained weight for the remainder of the study. By day 90, males and females given 750 or 1500 ppm weighed 8% or 9% and 3% or 8% less than controls, respectively. By day 90, the body weight gain of males and females given 750 or 1500 ppm was 11% or 13% and 9% or 20% less than that of controls, respectively. All body weight effects were considered secondary to the lower feed consumption owing to decreased palatability of the test material in the feed. Males and females at 100 ppm had body weights comparable to those of the controls at the end of the 90-day study. During the 28-day recovery period, feed consumption was comparable to that of controls in both sexes, and nearly complete recovery was seen in body weights (~5% lower for both sexes).

Serum cholesterol levels in males and females at 750 or 1500 ppm were increased by 51% or 127% and 32% or 83%, respectively (Table 15). Total protein and albumin levels were increased in high-dose males and females, but these were not considered to be of toxicological significance. Other clinical pathology values were comparable to control values. Following the 28-day recovery period, all clinical chemistry parameters in males and females at 1500 ppm were comparable to those of controls.

The absolute liver weights of males and females given 750 or 1500 ppm were 6% or 5% and 25% or 17% higher than controls, respectively, and the relative liver weights of males and females given 750 or 1500 ppm were 14% or 8% and 41% or 27% higher than controls, respectively (Table 15). There were a number of additional differences in organ weights of males and females

Table 15. Summary of selected findings in rats fed diets containing sulfoxaflor for 90 days

	Males				Females			
	Dietary concentration (ppm)							
	0	100	750	1500	0	100	750	1500
Dose (mg/kg bw per day), day 89	0	4.7	36.4	74.0	0	4.8	38.3	75.2
Plasma concentration (µg/g), day 89	0	1.5	12.0	24.1	0	1.3	10.1	20.2
Plasma $AUC_{24\,h}$ (µg·h/ml), day 89	0	35	281	555	0	30	235	476
Plasma $t_{½}$ (h), day 89	0	8.73	9.16	9.09	0	7.93	8.47	7.73
Cholesterol (mg/dl)	59	60	89*	134*	77	86	102*	141*
Total protein (g/dl)	6.9	6.7	7.1	7.5*	7.1	7.4	7.3	7.6*
Albumin (g/dl)	4.1	3.9	4.1	4.3#	4.4	4.5	4.4	4.6#
Body weight, terminal (g)	313.1	308.2	289.7	277.9*	168.8	170	164.6	155.3
Liver weight (g)	8.14	8.09	8.60	10.2*	4.20	4.36	4.40*	4.92*
Liver weight, relative (% of body weight)	2.60	2.62	2.97*	3.67*	2.49	2.56	2.67*	3.17*
Incidence of finding (*n* = 10)								
Hepatocytes, hypertrophy								
- very slight	0	0	0	0	0	0	9	5
- slight	0	0	7	1	0	0	0	5
- moderate	0	0	3	9	0	0	0	0
Hepatocytes, necrosis								
- very slight	0	0	8	5	0	0	3	10
- slight	0	0	2	5	0	0	0	0
Hepatocytes, vacuolation/fatty change								
- very slight	0	0	6	2	0	0	0	0
- slight	0	0	4	3	0	0	0	0
- moderate	0	0	0	5	0	0	0	0
Liver/aggregates of macrophages								
- very slight	9	10	7	4	10	10	10	8
- slight	1	0	3	6	0	0	0	2
Incidence after recovery period (*n* = 10)								
Hepatocytes, hypertrophy								
- very slight	0	—	—	2	—	—	—	0
Hepatocytes, necrosis								
- very slight	0	—	—	1	—	—	—	0
Hepatocytes, vacuolation/fatty change								
- very slight	0	—	—	7	—	—	—	0
- slight	0	—	—	1	—	—	—	0

From Yano et al. (2009c)

* $P < 0.05$ (Dunnett's test, males and females analysed separately); # $P < 0.05$ (Dunnett's test, males and females analysed together)

given 750 or 1500 ppm that were statistically significant. These differences in organ weights were secondary to the lower body weights of these dose groups and did not reflect a primary target organ effect of sulfoxaflor. This conclusion was supported by the absence of histopathological changes in these organs.

Treatment-related histological liver effects occurred in males and females given 750 or 1500 ppm and consisted of a dose-related increase in the severity (slight to moderate) of hepatocellular hypertrophy (with altered tinctorial properties) involving the centrilobular to midzonal regions of the hepatic lobule. Individual hepatocyte necrosis was also observed in the centrilobular region with a multifocal distribution to a very slight or slight degree (Table 15). All effects were seen in both sexes but were more prominent in males than in females. Vacuolation of hepatocytes, consistent with fatty change, was also observed in all males in the 750 and 1500 ppm groups at very slight, slight or moderate degrees. In addition, in the rats with the greatest degree of hepatocellular hypertrophy, necrosis and vacuolation, there was an increase in the incidence of multifocal aggregates of macrophages/histiocytes. The microscopic changes were present in all three lobes of the liver examined in male and female rats; however, they were more readily apparent in the right lateral lobe. The microscopic changes in the liver were consistent with the increased liver weights and cholesterol levels noted for these rats.

Following the 28-day recovery period, a complete recovery was seen in the absolute and relative liver weights of males and females given 1500 ppm. There was partial recovery of the microscopic hepatic effects. Two male rats in the 1500 ppm group still had recognizable hepatocellular hypertrophy of a very slight degree in the centrilobular and midzonal regions. One of these two rats also had multifocal, very slight individual hepatocellular necrosis. Multifocal, very slight or slight hepatocellular vacuolation consistent with fatty change was present in most of the recovery males; however, the degree of involvement was substantially less severe in the recovery group. There were no microscopic treatment-related changes present in the liver of females given 1500 ppm.

There was no effect on immune responsiveness in female rats up to and including the high dose level of 1500 ppm. In males, there was no effect on immune responsiveness at 100 or 750 ppm, whereas the 1500 ppm group displayed a lower, non-statistically significant response (26% lower) when compared with the vehicle control group. The lower AFC response in the high-dose male group coincided with considerable general toxicity, including decreased body weights and increases in liver weights (absolute and relative), hepatocellular hypertrophy, necrosis, vacuolation consistent with fatty change, multifocal aggregates of macrophages and elevated serum cholesterol, for which overall the NOAEL was 100 ppm. Therefore, the lower AFC response in the high-dose males was considered secondary to systemic toxicity and thus does not reflect primary immunotoxic potential for sulfoxaflor.

Toxicokinetic analysis of the plasma showed that the systemic exposure of sulfoxaflor was dose proportional, with an approximate 8-fold increase in $AUC_{24\,h}$ between the 100 and 750 ppm groups and an approximate 2-fold increase between the 750 and 1500 ppm doses. Females were more efficient than males at eliminating the test material from their system. The 24-hour systemic dose as measured by the plasma $AUC_{24\,h}$ was 15%, 16% and 14% lower in females than in males in the 100, 750 and 1500 ppm nominal dose groups, respectively. The plasma elimination half-life of sulfoxaflor in male rats was approximately 9 hours, whereas it was approximately 8 hours in females (12% lower). The chromatograms of the plasma samples taken from sulfoxaflor-dosed rats (via diet) contained up to five minor peaks in addition to the parent compound. These peaks may represent metabolites of the test material or metabolites of test material impurities. Absolute quantification of the minor metabolites was not possible, because of the lack of reference standards. Elimination of sulfoxaflor in urine over 24 hours ranged between 51% and 61% of the ingested dose, with the exception of high-dose males, from which 37% of the ingested dose was excreted, 26 days after the initiation of the study. Elimination of test material in 0- to 24-hour urine on days 84 and 85 ranged between 52% and 69% for the lower two dose levels, but was somewhat lower at the high dose for both sexes (32–36% of ingested dose). In addition to parent sulfoxaflor, four urinary metabolites were detected. One peak was a known impurity in this lot of the test material. No definitive quantification of the other three metabolites was obtained.

The NOAEL was 100 ppm (equal to 6.36 mg/kg bw per day), based on liver toxicity (increased serum cholesterol level, vacuolation/fatty change of hepatocytes) in males at 750 ppm (equal to 47.6 mg/kg bw per day) and above. All effects had recovered during the 28-day recovery phase, with the exception of very slight histopathological changes in the liver (vacuolation/fatty change of hepatocytes) in males at 1500 ppm (equal to 94.9 mg/kg bw per day) (Yano et al., 2009c).

In a study of dermal toxicity conducted in compliance with OECD test guideline 410, groups of 10 male and 10 female F344/DuCrl rats were administered sulfoxaflor (purity 95.6%) dermally at a dose level of 0, 100, 500 or 1000 mg/kg bw per day for 28 days (6 hours/day, 7 days/week; semi-occlusive dressing). Parameters evaluated were daily cage-side and weekly detailed clinical observations, dermal observations, ophthalmic examinations, body weight, feed consumption, haematology, clinical chemistry, urinalysis, toxicokinetics of blood plasma, selected organ weights, and gross pathological and histopathological examinations.

There were no mortalities, no treatment-related clinical observations and no ophthalmic findings at any dose level. No effects on body weights or on feed consumption were observed.

Serum cholesterol levels were slightly but statistically significantly increased (17%) in males exposed to 1000 mg/kg bw per day. This effect was considered non-adverse because it was marginal and was well within the laboratory's historical control range.

Absolute and relative liver weights of males exposed to 1000 mg/kg bw per day were marginally higher (6.5% and 4.4%, respectively) than those of the controls. Also, treatment-related histopathological changes were observed in the livers of 6 of 10 males exposed to 1000 mg/kg bw per day and consisted of very slight hepatocyte hypertrophy, with altered tinctorial properties (increased cytoplasmic eosinophilia), involving the centrilobular/midzonal regions of the hepatic lobule.

There were no treatment-related gross pathological or histopathological changes in the skin at the dermal test site in males or females exposed to doses up to and including 1000 mg/kg bw per day.

The results of toxicokinetic analysis showed that following dermal application, the average plasma concentration of the test material (< 0.14, < 0.14, 0.36 and 4.37 µg/g in males and < 0.14, < 0.14, 0.44 and 2.35 µg/g in females at 0, 100, 500 or 1000 mg/kg bw per day, respectively) at the high dose was greater than dose proportional. Similar plasma concentrations were found prior to and 16 hours after test material removal, indicating some retention of test material at the application site.

The NOAEL for systemic toxicity was 500 mg/kg bw per day, based on slight histopathological changes in the liver of males at 1000 mg/kg bw per day. There were no substance-related signs of local irritation up to 1000 mg/kg bw per day, the highest dose tested (Thomas, Murray & Saghir, 2009).

Dogs

In a palatability and tolerability pilot study, six male and three female Beagle dogs were given sulfoxaflor (purity 95.6%) by dietary or capsule administration using six different exposure procedures (phases). Phase A (six males, two females) evaluated the palatability of the test material in ground diet offered for 3 days at a concentration of 0, 300, 750 or 1500 ppm. Phase B (six males) evaluated the palatability of the test material in ground diet offered for 3 days at a concentration of 0, 300 or 750 ppm with bacon flavouring added. Phase C (four males, two females) evaluated the palatability of the test material in pre-formulated pelletized diet offered for 3 days at a concentration of 0, 300 or 750 ppm with bacon flavouring added. Phase D (four males) evaluated the tolerability of the test material administered in a gelatine capsule twice per day for 3 days at a dose level of 10 or 25 mg/kg bw per day (5 or 12.5 mg/kg bw per dose). Phase E (two males, one female) evaluated the palatability of the test material in ground diet offered for 2 hours/day for 2, 4 or 5 days, as dry diet or with water added to form a slurry, at respective concentrations of 50, 100 or 250 ppm. Phase F (six males, three females) evaluated the tolerability of the test material administered in a gelatine capsule after offering canned diet once per day for 11 days at a dose level of 10 or 15 mg/kg bw per day.

Observations for morbidity, mortality, injury and the availability of feed and water were conducted twice daily. Clinical observations were conducted as needed during the study. Body weights and feed consumption were measured and recorded daily during the treatment period of each phase. Ophthalmoscopic and physical examinations were conducted pretest. Blood samples for clinical pathology evaluations were collected pretest, prior to initiation of dosing of phase F and at the termination of phase F. Additional blood samples for clinical chemistry were also collected on day 8 of phase F. Urine and blood samples for potential evaluation of test material exposure were collected at the termination of phase F. Necropsy examinations were performed, liver weights were measured and recorded, and selected tissues were collected at the termination of phase F.

All animals survived each exposure phase. Individual clinical observations were associated with lack of palatability or intolerance to the test material. These observations included emesis, loss of skin elasticity, thinness and altered faecal appearance.

Feed consumption values during each test material exposure period were consistently well below acceptable intake limits (at least 75% of basal intake). In most cases, feed consumption values were as low as 10–20% of basal intake levels. Body weights were consistently decreased during test material exposure periods, which was directly associated with decreased feed consumption.

Erythrocytes, haemoglobin and haematocrit tended to decrease slightly in the females at 10 mg/kg bw per day at termination. Individual values remained within expected ranges, but reticulocytes in females at 10 mg/kg bw per day and in males at 15 mg/kg bw per day were markedly reduced, suggestive of potential suppressed erythropoiesis.

Moderate to marked dose-related decreases in sodium levels were present in males at 15 mg/kg bw per day at the interim and terminal intervals and in females at 10 mg/kg bw per day at termination relative to predose values and expected ranges. Decreases in chloride levels of similar magnitude were present in males and females at 10 mg/kg bw per day at the interim and terminal intervals and in males at 15 mg/kg bw per day at both intervals. Potassium levels also tended to decrease gradually over the duration of the study, with values below expected ranges at termination in males at both dose levels and in females at 10 mg/kg bw per day. These changes were attributed to the emesis and loose faeces noted throughout the study, suggesting indirect test article effects.

A dose-related increase in ALT activity was present in males at 15 mg/kg bw per day at termination and in females at 10 mg/kg bw per day at termination relative to expected ranges, which may suggest a treatment-related effect.

There were no treatment-related organ weight changes or macroscopic observations in males or females. Histopathological evaluation was not conducted for any phase of this study.

In conclusion, none of the exposure procedures of this palatability and tolerability study produced feed consumption results that were considered acceptable for repeated exposure over 28 or 90 days (Stewart, 2009a).

In a second palatability and tolerability study, six female Beagle dogs were given sulfoxaflor (purity 95.6% for technical grade, 99.7% for analytical grade) by dietary, capsule or oral gavage administration for up to 28 days. Using the same animals, routes of test material administration and dosing regimens were altered during the course of the study as requested by the sponsor, with a 1- or 2-week interval between regimens. Two groups of three female Beagle dogs were administered the test material as follows: One group of three animals received the test material via the diet, ad libitum for 6 consecutive days, at a dose level of 500 ppm (group 1). The dosing route for these animals was changed to oral gavage using analytical-grade sulfoxaflor, once daily for 28 consecutive days, at a dose level of 15 mg/kg bw per day (group 3). Another group of three animals received the test material via capsule, twice daily for 6 consecutive days, at a dose level of 15 mg/kg bw per day (group 2). The dosing route for these animals was changed to dietary, ad libitum for 5 consecutive days, at a

dose level of 100 ppm (group 4). The dosing route for these animals was once again changed to oral gavage using technical-grade sulfoxaflor, once daily for 28 consecutive days, at a dose level of 15 mg/kg bw per day (group 5). For both oral gavage groups, the vehicle was 0.5% methylcellulose in deionized water, and the dose volume was 10 ml/kg bw.

Observations for morbidity, mortality, injury and the availability of feed and water were conducted twice daily for all animals. Clinical observations were conducted daily. Body weights and feed consumption were measured and recorded daily. Blood samples for clinical pathology evaluations were collected from all animals pretest and on days 6 and 29 (prior to necropsy), and urine samples were collected at necropsy from all animals on day 29. Blood samples for determination of the plasma concentrations of the test article were collected from animals in group 4 at designated time points on day 5 (the last day of dietary dosing) and from animals in groups 3 and 5 at designated time points on each respective day 28 (prior to necropsy). At study termination, necropsy examinations were performed and organ weights were recorded. A complete set of tissues from group 5 animals was sent to the sponsor for microscopic evaluation.

All animals survived until the scheduled termination intervals. However, there were clinical findings and body weight findings that were associated with reduced feed consumption. In some instances, the reduced feed consumption was accompanied by a lack of sufficient fluid intake, which resulted in a loss of skin elasticity. In addition, intermittent emesis and faecal alterations (discoloured, mucoidal and soft/watery faeces) were likely treatment related.

All exposures resulted in decreases in feed consumption and body weight, with the exception of dietary exposure at 100 ppm. However, feed consumption and body weights during oral gavage exposure tended to stabilize after an initial decrease.

There were no treatment-related effects on haematology, clinical chemistry or urinalysis parameters, with the possible exception of low urine volumes at 15 mg/kg bw per day (both groups).

The only organ weight alteration that was likely related to treatment was the lower thymus weights of all group 3 animals; however, no histopathological examination was conducted.

There were no treatment-related gross pathological observations in group 5 animals, and no treatment-related histopathological changes were observed.

Toxicokinetic analysis showed that the orally gavaged sulfoxaflor was rapidly absorbed from the gastrointestinal tract without any lag time and appeared in blood within 15 minutes of dosing. The peak plasma concentration (C_{max}) of sulfoxaflor was reached within 4 ± 2 hours after dosing. Elimination of sulfoxaflor from plasma was slow (elimination half-life = 26 ± 18 hours and clearance = 18 ± 13 ml/kg bw per hour). The daily systemic dose ($AUC_{24\,h}$) of sulfoxaflor to dogs after multiple gavage dosing at 15 mg/kg bw per day was 479 ± 111 µg·h/ml, and the dose-corrected $AUC_{24\,h}$ was 32 ± 7 µg·h/ml per milligram per kilogram body weight administered.

In conclusion, oral gavage administration at 15 mg/kg bw per day was a tolerable route of exposure and concentration for the technical-grade test material exposure, as determined by adequate feed consumption and tolerable in-life clinical signs. Also, exposure at 100 ppm via the dietary route was well tolerated, as determined by adequate feed consumption, but the amount of test material consumed was not sufficient to justify it as a potential high dose level for subsequent studies.

Exposure at 500 ppm via the dietary route or 15 mg/kg bw per day via the capsule route was not well tolerated, as determined by insufficient feed consumption. Therefore, these concentrations and routes could not be considered a viable method of test material exposure over a sustained duration. Based on this information, oral gavage exposure was determined to be the most appropriate route for a sustained duration of test material exposure in Beagle dogs (Stewart, 2009b).

In a study of toxicity conducted according to OECD test guideline 409, groups of four male and four female Beagle dogs were administered sulfoxaflor (purity 95.6%) by oral gavage in 0.5%

aqueous methylcellulose (Methocel A4C) at a dose level of 0, 1, 3 or 10 mg/kg bw per day for 90 days. However, on day 5, the highest dose level was reduced to 6 mg/kg bw per day because of intolerance (as determined by a lack of feed consumption). The dose volume was 10 ml/kg bw, except from day 5 to day 8, when the 10 mg/kg bw per day dose was administered at 6 ml/kg bw to achieve the 6 mg/kg bw per day dose.

Observations for morbidity, mortality, injury and the availability of feed and water were conducted twice daily for all animals. Clinical observations were conducted and body weights were measured and recorded weekly. Feed consumption was measured daily from days 1 to 7 and days 9 to 35, at least twice weekly from weeks 6 to 12 and at least once during week 13. Physical examinations were conducted by a veterinarian pretest to confirm the good health status of each animal placed on study. Blood and urine samples for clinical pathology evaluations were collected from all animals pretest and during weeks 6 and 13. Blood and urine samples were collected from all animals at designated intervals during week 13 for determination of the plasma and urine concentrations of the test material. At study termination, necropsy examinations were performed, organ weights were recorded and selected tissues were examined microscopically.

There were no mortalities and no treatment-related clinical observation findings at any dose level.

Treatment-related decreased mean feed consumption values were observed at the high dose level during the first 2 weeks of the study. Beginning in week 3, the mean feed consumption values at this exposure level stabilized and were similar to control values. Based on the data, the effect on feed consumption was considered to be related to exposure at 10 mg/kg bw per day, with residual effects prolonging the instability into week 2 of the study. There were no other notable feed consumption findings for the remainder of the study.

Treatment-related decreased mean body weights were observed at 10 mg/kg bw per day during the 1st week of exposure. However, mean body weights at this exposure level did not decrease significantly beyond week 1, effectively after the dose was reduced to 6 mg/kg bw per day on day 5, and by approximately week 9, the mean body weights at this exposure level had returned to pre-exposure values. The transient nature of the decreased mean body weights was a clear indication that the effect was related to exposure at 10 mg/kg bw per day and not related to exposure at 6 mg/kg bw per day.

There were no treatment-related effects on haematology, clinical chemistry or urinalysis parameters.

There were no treatment-related gross pathological observations, organ weight changes or histopathological changes at any exposure level.

The toxicokinetic analysis after 88 days of dosing at 1, 3 or 6 mg/kg bw per day showed that the daily systemic dose ($AUC_{24\,h}$) was 32 ± 6, 84 ± 23 and 147 ± 13 µg·h/ml in males and 22 ± 3, 71 ± 26 and 119 ± 19 µg·h/ml in females, respectively. The increase in systemic dose was clearly dose proportional in female dogs and was approximately dose proportional in male dogs across all three dose levels. The dose-corrected $AUC_{24\,h}$ was 32, 28 and 25 µg·h/ml per milligram per kilogram body weight administered in males and 22, 24 and 20 µg·h/ml per milligram per kilogram body weight administered in females, respectively. The mean plasma elimination half-life of sulfoxaflor at 1, 3 or 6 mg/kg bw per day was 20.3 ± 4.2, 28.2 ± 7.1 and 17.1 ± 6.1 hours in males and 27.5 ± 26.1, 20.9 ± 8.5 and 16.9 ± 1.9 hours in females, respectively.

Urinary elimination of sulfoxaflor was 70% ± 6%, 76% ± 12% and 59% ± 33% of the administered dose in male dogs and 69% ± 4%, 80% ± 6% and 74% ± 13% in female dogs at 1, 3 and 6 mg/kg bw per day, respectively.

The NOAEL was 6 mg/kg bw per day, based on decreased feed consumption and decreased body weights during the 1st week of exposure at 10 mg/kg bw per day. The reduction of this initial high dose to 6 mg/kg bw per day on day 5 allowed for recovery, and no treatment-related adverse effects occurred at any dose level thereafter (Stewart, 2010).

In a study of toxicity conducted according to OECD test guideline 452, groups of four male and four female Beagle dogs were administered sulfoxaflor (purity 95.6%) by oral gavage in 0.5% aqueous methylcellulose (Methocel A4C) at a dose level of 0, 1, 3 or 6 mg/kg bw per day for 1 year. The dose volume was 10 ml/kg bw. Observations for morbidity, mortality, injury and the availability of feed and water were conducted twice daily for all animals. Toxicity was assessed by weekly detailed clinical observations, feed consumption and body weight measurements, ophthalmoscopic examinations and clinical pathology evaluations. Blood and urine samples for the determination of the plasma concentrations of the test material were collected from all animals at designated time points during weeks 13, 26 and 52, and systemic exposure was determined. The toxicokinetic parameters were determined for the test article from concentration–time data. At study termination, necropsy examinations were performed, select organ weights were recorded and tissues were preserved for subsequent microscopic examination.

No treatment-related deaths occurred. An increase in the incidence of both soft and watery faeces occurring in two males (animal numbers 125 and 126) at 6 mg/kg bw per day was considered treatment related; the other two high dose level males were normal in this respect. A transient, treatment-related decrease in feed consumption and body weight was observed in two females (animal numbers 129 and 132) at 6 mg/kg bw per day during the first 2 weeks of dosing (Table 16).

One male at 6 mg/kg bw per day (animal number 125) exhibited increased ALP, total bilirubin, triglycerides, total cholesterol, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol at 3 months. The triglyceride levels remained within expected ranges, but were increased relative to controls and other animals within the 6 mg/kg bw per day group. These changes were decreasing at 6 months and resolved at 12 months. Whether these were transient treatment-related effects to which there was adaptation or were incidental cannot be clearly ascertained. No other treatment-related effects on clinical pathology parameters were noted.

There were no treatment-related macroscopic findings, effects on organ weights or histopathological effects at any dose.

The systemic exposure to sulfoxaflor ($AUC_{24\ h}$) in plasma was proportional across all dose levels in both sexes. Except in female dogs at 52 weeks of exposure, toxicokinetic analysis of parent compound in urine showed that the systemic exposure to sulfoxaflor was proportional across all dose levels and time points. The deviation in females at 52 weeks was relatively modest.

The NOAEL was 6 mg/kg bw per day, the highest dose tested. The increased incidences of soft/watery faeces in two males at 6 mg/kg bw per day were not considered adverse, as these changes were not accompanied by any other toxicological effect. Also, the slight decreases in feed consumption and body weight in two females at 6 mg/kg bw per day during the first 2 weeks of dosing were not considered adverse, as there were no changes during the remainder of the study (Heward, 2010a).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a study of carcinogenicity conducted according to OECD test guideline 451, groups of 50 male and 50 female Crl:CD1(ICR) mice were given diets containing sulfoxaflor (purity 95.6%) at a concentration of 0, 25, 100 or 750 ppm in males (equal to 0, 2.54, 10.4 and 79.6 mg/kg bw per day) and at a concentration of 0, 25, 250 or 1250 ppm in females (equal to 0, 3.43, 33.9 and 176 mg/kg bw per day) for up to 18 months. Animals were evaluated by daily cage-side, biweekly clinical observations, periodic hand-held detailed clinical examinations, body weights and feed consumption. Ophthalmic examinations were conducted pre-exposure and prior to necropsy. All mice had a complete necropsy examination with total and differential white blood cell counts and weights of selected organs at the scheduled necropsy. An extensive set of organs was examined histopathologically from all control and high dose group mice, as well as all mice that died or were euthanized in moribund

Table 16. Summary of selected findings in dogs fed diets containing sulfoxaflor for 1 year

	Males				Females			
	Dose (mg/kg bw per day)							
	0	1	3	6	0	1	3	6
Plasma $AUC_{24\,h}$ (µg·h/ml)								
- week 13	0	30	95	167	0	27	75	155
- week 26	0	31	83	146	0	27	76	165
- week 52	0	30	92	182	0	29	82	204
Faeces soft[a]	8/3	27/4	10/3	271/4	23/3	16/4	44/4	31/3
Faeces watery[a]	0/0	10/2	1/1	315/2	8/3	4/2	29/3	24/3
Body weight (kg)								
- week −1	9.36	9.40	9.63	9.33	7.60	7.71	7.77	7.70
- week 1	9.41	9.56	9.56	9.23	7.84	7.84	7.65	7.58
- week 2	9.62	9.86	9.62	9.23	7.86	7.87	7.77	7.60
- week 52	11.31	11.47	10.93	10.79	8.84	8.89	8.83	9.21
Feed consumption (g/animal per day)								
- week 1	231	275	199	188	253	242	209	155
- week 2	237	293	211	203	224	228	240	191

From Heward (2010a)

[a] Number of times observed/number of animals affected.

condition. The kidneys, liver, lungs, adrenal glands (females only) and all relevant gross lesions of mice from the low- and intermediate-dose groups from the terminal necropsy were also examined histopathologically.

There were no treatment-related changes in clinical observations, body weights and body weight gains, feed consumption, ophthalmological observations, or total and differential white blood cell counts in any of the sulfoxaflor-treated groups.

Toxicokinetic analysis indicated that the daily systemic dose (steady-state plasma concentration) of sulfoxaflor in male and female mice remained essentially proportional to dose during the course of the study (3 and 12 months after study start), and concentrations of sulfoxaflor in urine also increased proportionally with dose for both male and female mice and at both collection times (3 and 12 months).

The liver was the primary target of sulfoxaflor. The absolute and relative liver weights of males given 750 ppm were increased by 87% and 79%, respectively; in females given 1250 ppm, they were increased by 51% and 47%, respectively, compared with their respective controls, which also reflected a treatment-related increased incidence of liver tumours, as described below. At necropsy, there was a treatment-related increase in the incidence of mass nodules and multifocal pale foci in the liver of males given 750 ppm, compared with controls. Females given 1250 ppm had a treatment-related increased incidence of mass nodules in the liver, albeit at lower numbers compared with the high-dose males. Histopathological treatment-related changes consisted of a statistically significant increase in the incidences of both hepatocellular adenomas and carcinomas in male mice given 750 ppm, with 60% of such animals exhibiting hepatocellular carcinomas and/or adenomas. There was no effect at 100 ppm. The incidence in female mice given 1250 ppm was 10%, compared with 2% in controls, although this difference was not statistically significant. Although there were no statistically identified differences in the overall mortality rates between the controls and any of the sulfoxaflor-treated groups, hepatocellular carcinomas or adenomas were attributed as the cause of death or moribundity in a small proportion of

Table 17. Summary of selected findings in mice fed diets containing sulfoxaflor for up to 18 months

	Males				Females			
	Dietary concentration (ppm)							
	0	25	100	750	0	25	250	1250
Dose (mg/kg bw per day), 3 months	0	2.8	10.6	89.6	0	3.7	45.2	197
Plasma concentration (µg/g), 3 months	0	0.7	2.7	18.2	0	0.6	5.6	28.3
Dose (mg/kg bw per day), 12 months	0	2.2	8.3	64.8	0	3.0	30.7	144
Plasma concentration (µg/g), 12 months	0	0.8	2.9	21.4	0	0.7	5.0	29.1
Terminal body weight (g)	46.9	50.0	47.9	48.0	39.0	41.9	41.8	40.1
Liver weight, absolute (g)	2.54	2.48	3.72	4.75*	1.89	2.72	2.07	2.86*
Liver weight, relative (% of body weight)	5.44	4.99	7.40	9.75*	4.882	5.95	5.02	7.21*
Liver, histopathology								
No. examined	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*
Foci, basophilic	3	1	4	2	0	0	1	0
Foci, eosinophilic	3	2	3	10	0	0	0	0
Foci, vacuolated	1	0	1	6	0	0	0	0
Hypertrophy, centrilobular	6	8	7	35*	2	0	14	29*
Hypertrophy, panlobular	0	0	0	12*	3	2	4	12*
Necrosis, multifocal	9	4	3	28*	1	3	1	6
Vacuolation, fatty change	2	1	1	16*	1	0	0	5
Mitotic alteration	5	1	1	10	1	3	2	0
Adenoma, hepatocyte	12	6	10	24*	1	1	0	2
Carcinoma, hepatocyte	2	0	4	17*	0	1	0	4
Adenoma and/or carcinoma	13	6	12	30*	1	2	0	5

From Thomas et al. (2010b)
* $P < 0.05$

males (6/50) given 750 ppm. Treatment-related non-neoplastic liver effects consisted of increases in the incidences of eosinophilic and vacuolated foci of cellular alteration in males given 750 ppm; slight to moderate centrilobular/midzonal or panlobular hepatocellular hypertrophy, with altered tinctorial properties (increased cytoplasmic eosinophilia), consistent with liver enzyme induction in males and females given 750 or 1250 ppm; multifocal individual cell necrosis of hepatocytes in males given 750 ppm (very slight or slight) and females given 1250 ppm (very slight); very slight fatty change in centrilobular/midzonal hepatocytes in males and females given 750 or 1250 ppm; and increased incidence of hepatocytes in mitosis in males given 750 ppm (Table 17).

The only treatment-related change in females given 250 ppm was an increased incidence of slight centrilobular/midzonal hepatocyte hypertrophy, with altered tinctorial properties (increased cytoplasmic eosinophilia), consistent with liver enzyme induction. This was considered to be an adaptive and non-adverse response due to a lack of any associated changes, including increase in liver weight or any other treatment-related histopathological findings.

Males given 750 ppm had an exacerbation in the cumulative incidence of spontaneous dermatitis, which is common in CD-1 mice. Histologically, this was characterized by subacute to chronic inflammation, variable epidermal ulceration and acanthosis. Associated with the ulcerative dermatitis was an increased incidence of reactive plasmacytosis of the local submandibular lymph nodes of males given 750 ppm. The exacerbation of spontaneous dermatitis in males given 750 ppm

was interpreted as secondary to the excessive stress induced by the malignant and/or benign hepatocellular neoplasms.

The NOAEL for carcinogenicity was 100 ppm (equal to 10.4 mg/kg bw per day), based on an increased incidence of hepatocellular adenomas and/or carcinomas in males at 750 ppm (equal to 79.6 mg/kg bw per day). The NOAEL for non-neoplastic changes was 100 ppm (equal to 10.4 mg/kg bw per day), based on liver toxicity (vacuolation/fatty change of hepatocytes) in males at 750 ppm (equal to 79.6 mg/kg bw per day) (Thomas et al., 2010b).

Rats

In a combined study of chronic toxicity and carcinogenicity conducted according to OECD test guideline 453, groups of 60 male and 60 female F344/DuCrl rats were given diets containing sulfoxaflor (purity 95.6%) at a concentration of 0, 25, 100 or 500 ppm in males (equal to 0, 1.04, 4.24 and 21.3 mg/kg bw per day) and at a concentration of 0, 25, 100 or 750 ppm in females (equal to 0, 1.28, 5.13 and 39.0 mg/kg bw per day) for up to 24 months. Ten rats of each sex per dose level were necropsied after 1 year (chronic toxicity group), and the remaining 50 rats of each sex per dose level were fed the respective diets for up to 2 years.

Animals were evaluated by daily cage-side examinations, biweekly clinical observations, periodic detailed clinical observations, body weights, feed consumption and toxicokinetics (plasma and urine). Clinical pathology examinations (haematology, clinical chemistry and urinalysis) were conducted at regular intervals throughout the study. Ophthalmic examinations were conducted on all rats pre-study and on all surviving rats prior to the scheduled necropsies. All rats had a complete necropsy, with weights of multiple organs collected from all rats at the scheduled necropsies. Histopathological examination of an extensive set of organs was performed on all control and high dose level rats and all rats that died spontaneously or were euthanized due to their moribund condition. Histopathological examination of rats from the low and intermediate dose levels of the 12-month chronic toxicity group was limited to the liver, adrenal glands, testes and relevant gross lesions. Histopathological examination of survivors from the low and intermediate dose levels of the 24-month oncogenicity group was limited to the liver, testes, epididymides, coagulating glands, prostate, seminal vesicles and relevant gross lesions.

During the study, there were no treatment-related clinical signs, and there were no statistical differences in mortality for either males or females at any dose level.

Males given 500 ppm had statistically significantly lower body weights at most measurement intervals starting on day 512 and continuing through day 729. On day 729, the mean body weight and body weight gain for males given 500 ppm were 5.0% and 5.7% lower than those of controls, respectively. Females given 750 ppm had statistically significantly lower body weights, first noted on day 57 and continuing through day 729. On day 729, the mean body weight and body weight gain for females given 750 ppm were 6.3% and 9.1% lower than those of controls, respectively. The body weights of males and females given 100 or 25 ppm were unaffected by treatment with sulfoxaflor (Table 18).

High dose level males and females had statistically significantly higher cholesterol concentrations at 3, 6 and 12 months and at 3, 6, 12 and 18 months, respectively, with increases ranging from 17.5% to 32.9%, which exceeded the respective historical control ranges. Values in both males and females at 24 months were similar to those in control animals.

The liver was the primary target organ for histopathological effects in males given 500 ppm and in females given 750 ppm at 12 and 24 months. The absolute and relative liver weights for high-dose males (500 ppm) and females (750 ppm) were increased at 12 months, in the range of 3.2–17% (Table 19), and relative weights were also statistically significantly increased at 24 months (Table 20). Non-neoplastic liver effects at 12 and 24 months consisted of hypertrophy of centrilobular and midzonal hepatocytes, necrosis of individual centrilobular hepatocytes, vacuolation (consistent with

Table 18. Summary of selected findings in rats fed diets containing sulfoxaflor for up to 2 years

	Males				Females			
	Dietary concentration (ppm)							
	0	25	100	500	0	25	100	750
Dose (mg/kg bw per day), 3 months	0	1.2	4.7	25.2	0	1.4	5.9	42.0
Plasma concentration (µg/g), 3 months	0	0.7	2.7	14.0	0	0.7	2.6	19.1
Dose (mg/kg bw per day), 12 months	0	0.9	3.8	19.1	0	1.3	5.2	35.8
Plasma concentration (µg/g), 12 months	0	0.4	1.8	9.8	0	0.6	2.2	19.1
Plasma $AUC_{24\,h}$ (µg·h/ml), 12 months	0	9.7	42.1	228	0	12.7	50.8	422
Mortality (no. of animals), 24 months	16	18	21	17	12	10	13	6
Body weight (g)								
- day 176	372.8	375.0	369.8	372.0	200.6	199.1	203.6	193.2*
- day 365	432.7	435.4	433.7	429.1	233.1	230.6	237.1	221.8*
- day 624	463.0	465.4	454.8	442.3*	291.4	289.1	299.9	274.3*
- day 729	439.3	443.2	418.6	417.4*	295.4	293.6	305.0	276.8*
Cholesterol (mg/dl)								
- 3 months	59	64	62	77*	93	101	103*	118*
- 6 months	72	78	74	85*	116	112	121	138*
- 12 months	79	95*	89	105*	143	137	140	168*
- 18 months	111	131	116	127	133	134	124	158*

From Stebbins et al. (2010a)
* $P < 0.05$

fatty change) of hepatocytes and an increase in the severity of aggregates of macrophages/histiocytes. An additional treatment-related liver effect in females given 750 ppm at 24 months consisted of a lower number of basophilic foci of altered hepatocytes (quantified as 21 or more basophilic foci in the three standard liver sections examined microscopically). A treatment-related neoplastic liver effect at 24 months consisted of a statistically significant increase in the incidence of benign hepatocellular adenomas in males given 500 ppm. Females given 750 ppm did not have any change in the incidence of liver tumours. There were no treatment-related liver effects in males or females given 25 or 100 ppm (Tables 19 and 20).

At 24 months, males given 100 or 500 ppm had treatment-related statistically significant higher absolute and relative testes weights. Absolute testes weights of males given 100 or 500 ppm were approximately 46% and 62% higher than those of controls, respectively. The higher testes weights were reflective of larger interstitial (Leydig) cell adenomas in the testes of males at these dose levels (Table 20).

The incidence of interstitial cell adenomas in at least one testis of males from all dose groups was comparable to that of controls at 24 months. However, males given 500 ppm had a statistically significant increase in the incidence of bilateral interstitial cell adenomas of the testes and a corresponding decrease in the incidence of unilateral interstitial cell tumours, relative to controls. An additional treatment-related testicular effect consisted of a statistically significant increase in the incidence of severe bilateral atrophy of seminiferous tubules in males given 100 or 500 ppm (Table 20).

At 24 months, males given 100 or 500 ppm had treatment-related statistically significantly lower absolute and relative epididymal weights. The lower epididymal weights were associated with a higher incidence of decreased spermatic elements (bilateral, severe) in the lumen of the epididymides of these males.

Table 19. Summary of selected morphological findings in rats at 12 months

	Males				Females			
	Dietary concentration (ppm)							
	0	25	100	500	0	25	100	750
Body weight (g)	391.5	416.5	410.3	404.0	214.2	215.8	216.3	207.9
Liver weight, absolute (g)	9.43	10.19	10.01	11.04*	5.59	5.50	5.69	5.77
Liver weight, relative (% of body weight)	2.41	2.44	2.44	2.73*	2.62	2.55	2.63	2.78*
Hepatocytes, hypertrophy (no.)								
- very slight	0	0	0	2	0	0	0	8
- slight	0	0	0	6	0	0	0	0
- moderate	0	0	0	2	0	0	0	0
Hepatocytes, necrosis (no.)								
- very slight	1	0	0	7	0	0	0	3
- slight	0	0	0	1	0	0	0	0
Hepatocytes, vacuolization (no.)								
- very slight	6	10	9	2	4	2	6	5
- slight	0	0	0	7	0	0	0	4
Testes; interstitial cell, hyperplasia, bilateral (no.)	9	8	10	10	—	—	—	—
Testes, interstitial cell adenoma; unilateral (no.)[a]	0	1	3	3	—	—	—	—

From Stebbins et al. (2010a)
* $P < 0.05$
[a] Historical control incidences (12-month sacrifice) from six studies: testicular interstitial cell adenomas: 0/10 to 3/10 (0–30%).

Table 20. Summary of selected morphological findings in rats at 24 months

	Males				Females			
	Dietary concentration (ppm)							
	0	25	100	500	0	25	100	750
Terminal body weight (g)	415.2	418.4	396.0	394.2	278.2	275.4	283.4	257.2*
Liver weight, absolute (g)	12.15	11.87	12.06	12.02	7.59	7.33	7.79	7.67
Liver weight, relative (% of body weight)	2.99	2.86	3.08	3.08*	2.75	2.66	2.75	2.99*
Testes weight, absolute (g)	3.72	3.93	5.42*	6.03*	—	—	—	—
Testes weight, relative (% of body weight)	0.91	0.94	1.36*	1.52*	—	—	—	—
Epididymides weight, absolute (g)	0.56	0.49	0.43*	0.41*	—	—	—	—
Epididymides weight, relative (% of body weight)	0.14	0.12	0.11*	0.11*	—	—	—	—
Liver								
No. examined	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*
Aggregates of macrophages								
- very slight	32	40	26	27	38	38	34	26*
- slight	4	1	4	16*	4	0	8	21*
Foci of cellular alteration, basophilic								
- 1–5	17	15	13	18	2	4	3	9
- 6–10	22	14	14	23	7	3	7	17*
- 11–20	5	12	11	1	19	21	18	18
- > 20	0	1	1	0	19	18	18	2*

Table 20 (continued)

	Males				Females			
	Dietary concentration (ppm)							
	0	25	100	500	0	25	100	750
Foci of cellular alteration, eosinophilic								
- 1–5	26	33	26	20	26	32	30	36
- 6–10	7	2	7	11	0	0	1	2
- 11–20	2	0	3	7	0	1	0	0
- > 20	0	0	0	1	0	0	0	0
Hepatocellular hypertrophy								
- very slight	2	0	0	0	0	1	0	33*
- slight	0	0	0	34*	0	0	0	5
Hepatocellular necrosis								
- very slight	2	0	0	24*	0	0	1	22*
- slight	0	0	0	1	0	0	0	1
Hepatocellular vacuolation/fatty change								
- very slight	21	25	21	23	27	35	36	9*
- slight	17	8	12	20	9	7	5	28*
- moderate	1	1	2	0	0	0	1	7*
Hepatocellular adenoma	4	2	5	16*	3	2	2	2
Hepatocellular carcinoma	3	1	1	0	0	0	0	0
Hepatocellular adenoma/carcinoma	7	3	6	16	3	2	2	2
Testes								
No. examined	*50*	*50*	*50*	*50*	—	—	—	—
Seminiferous tubule, atrophy, bilateral	13	15	25*	34*	—	—	—	—
Interstitial cell hyperplasia								
- unilateral	1	2	0	2	—	—	—	—
- bilateral	4	0	3	2	—	—	—	—
Interstitial cell adenoma								
- unilateral[a]	12	8	5	2*	—	—	—	—
- bilateral[a]	32	38	40	44*	—	—	—	—
- unilateral/bilateral[a]	44	46	45	46	—	—	—	—
Epididymides								
No. examined	*50*	*50*	*50*	*50*	—	—	—	—
Decreased spermatic elements, bilateral	21	23	29	37*	—	—	—	—
Coagulating gland								
No. examined	*50*	*50*	*50*	*50*	—	—	—	—
Decreased secretory material	10	11	16	21*	—	—	—	—
Prostate								
No. examined	*50*	*50*	*50*	*50*	—	—	—	—
Decreased secretory material	13	15	17	25*	—	—	—	—
Seminal vesicle								
No. examined	*50*	*50*	*50*	*50*	—	—	—	—
Decreased secretory material	10	11	16	21*	—	—	—	—
Preputial gland								
No. examined	*8*	*8*	*7*	*10*	—	—	—	—
Carcinoma	5	7	7	10	—	—	—	—

From Stebbins et al. (2010a)

* $P < 0.05$

[a] Historical control incidences (24-month sacrifice) from five studies conducted in 2004–2009: interstitial cell adenomas, unilateral: 4–8 (8–16%); interstitial cell adenomas, bilateral: 32–39 (64–78%); interstitial cell adenomas, unilateral or bilateral: 38–46 (76–92%).

Males given 500 ppm had statistically significant increases in the incidence of decreased secretory material in the coagulating glands (severe), prostate (moderate) and seminal vesicles (severe) at 24 months. Males given 500 ppm also had an increased incidence of carcinoma of the preputial gland that was interpreted to be treatment related.

Toxicokinetic analyses of plasma samples at 3 and 12 months indicated dose proportionality in systemic dose levels that were comparable between the two time points. There were no sex differences in plasma concentrations of sulfoxaflor across the dose levels and times analysed. Urinary elimination of sulfoxaflor was also dose proportional for both male and female rats at 3, 6 and 12 months, representing 58–127% of the average test material consumed in a 24-hour period.

Although the mode of action (MOA) for the male reproductive organ effects was not investigated as part of this study, the primary effect was interpreted to be larger interstitial cell adenomas. The effects on seminiferous tubules, epididymides, accessory sex glands and preputial gland were thought to be secondary to loss of normal testicular function due to the large size of the interstitial cell adenomas and possible alterations in the endocrine balance of these rats, as there was a high correlation between the presence of large interstitial cell adenomas and effects noted in the testicular seminiferous tubules, epididymides and accessory sex glands. In regards to severe bilateral atrophy of testicular seminiferous tubules, there was 100% correlation of this finding with the presence of large interstitial cell adenomas (adenomas that occupied approximately 75% or more of the cross-sectional area of the testes). The majority of control and treated males that had severe bilateral decreased spermatic elements of the epididymides, severe bilateral decreased secretory material of the coagulating glands and seminal vesicles and moderate bilateral decreased secretory material of the prostate had large interstitial cell adenomas. These correlations, noted in control and treated males, suggest that the enlargement of the interstitial cell adenomas is the primary effect, and the other effects are secondary to loss of normal testicular function due to the overwhelming size of the interstitial cell adenomas, and not primary responses to administration of sulfoxaflor.

The NOAEL for carcinogenicity was 100 ppm (equal to 4.24 mg/kg bw per day), based on an increased incidence of hepatocellular adenomas in males at 500 ppm (equal to 21.3 mg/kg bw per day). Also at 500 ppm, there was an increased incidence of bilateral Leydig (interstitial) cell adenomas of the testes, whereas there was no effect on the incidence of combined unilateral/bilateral Leydig cell adenomas. The size and weight of the testes and the size of Leydig cell adenomas were increased at 100 and 500 ppm and were associated with the secondary changes in the testes and epididymides listed below.

The NOAEL for non-neoplastic effects was 25 ppm (equal to 1.04 mg/kg bw per day), based on changes in the testes (increased testes weights, increased incidence of severe bilateral atrophy of seminiferous tubules) and epididymides (decreased epididymal weights, increased incidence of severe bilateral decreased spermatic elements of the epididymides) in males at 100 ppm (equal to 4.24 mg/kg bw per day) and above.

In females, the NOAEL for non-neoplastic effects was 100 ppm (equal to 5.13 mg/kg bw per day), based on degenerative changes in the liver (vacuolation/fatty change of hepatocytes) at 750 ppm (equal to 39.0 mg/kg bw per day) (Stebbins et al., 2010a).

2.4 Genotoxicity

Genotoxicity studies with sulfoxaflor are summarized in Table 21.

In a reverse gene mutation assay in bacteria conducted according to OECD test guideline 471 (1997), *Salmonella typhimurium* (strains TA98, TA100, TA1535, TA1537) and *Escherichia coli* (strain WP2*uvr*A) were exposed to sulfoxaflor (purity 96.6%), using DMSO as solvent, in the

Table 21. Summary of genotoxicity studies with sulfoxaflor

End-point	Test object	Concentration or dose	Purity (%)	Result	Reference
In vitro					
Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537	± S9 mix: 0–5000 µg/plate	96.6	Negative	Mecchi (2007)
	Escherichia coli WP2*uvr*A				
Gene mutation, *HPRT* locus	Chinese hamster ovary cells	± S9 mix: 0, 173.3–2773 µg/ml	96.6	Negative	Schisler, Geter & Trombley (2007)
Chromosomal aberration	Rat lymphocytes (whole blood)	± S9 mix: 0, 173.3–2773 µg/ml	96.6	Negative	Schisler, Geter & Kleinert (2007)
In vivo					
Micronucleus induction	Male and female Crl:CD1(ICR) mice, bone marrow erythroblasts	0, 100, 200 and 400 mg/kg bw; twice (24 h apart); oral administration	95.6	Negative	LeBaron & Schisler (2009)

S9, 9000 × *g* rat liver supernatant

presence and absence of S9 metabolic activation in two independent sets of experiments. Doses were selected based on the results of a range-finding study. For the initial preincubation test using doses of up to and including 5000 µg/plate, three plates were used for each strain, condition and dose. Vehicle and positive controls were included in each experiment. The independent repeat utilized the same conditions as the initial test. Doses up to and including 5000 µg/plate did not cause any bacteriotoxic effects. Total bacteria counts remained unchanged, and no inhibition of growth was observed.

No evidence for mutagenic activity of sulfoxaflor was seen. No biologically relevant increase in the mutant count, in comparison with the negative controls, was observed. The positive controls induced the appropriate responses in the corresponding strains. Therefore, sulfoxaflor was considered to be not mutagenic in the bacterial strains tested, in either the presence or absence of metabolic activation (Mecchi, 2007).

In an in vitro mammalian cell gene mutation test conducted according to OECD test guideline 476 (1997), sulfoxaflor (purity 96.6%) dissolved in DMSO was tested for its ability to induce forward mutations at the *HPRT* locus in Chinese hamster ovary (CHO) cells. Two independent sets of experiments were conducted in the presence and absence of S9 metabolic activation. Based on the guideline limit dose (10 mmol/l), concentrations of 173.3–2773 µg/ml were used in the main study both with and without metabolic activation, and the same concentrations were used for the independent repeats. Ethyl methanesulfonate (EMS) and 20-methylcholanthrene (20-MCA) served as positive controls in the experiments without and with metabolic activation, respectively. The cells were treated for 4 hours in both experiments, without and with metabolic activation. After this, the incubation media were replaced by culture medium, and the cells were incubated (and subcultured every 2–3 days) for about 7–9 days for expression of mutant cells. This was followed by incubation of cells for 6–10 days in selection medium containing 6-thioguanine.

Neither in the initial nor in the confirmatory study was any increase in the mutant frequency observed. In contrast to this, the positive control substances EMS and 20-MCA resulted in a marked increase in mutant frequency. Based on the results of the study, sulfoxaflor was considered to be not mutagenic in the CHO/HPRT forward mutation assay, in either the presence or absence of metabolic activation (Schisler, Geter & Trombley, 2007).

In an in vitro mammalian chromosomal aberration test conducted according to OECD test guideline 473 (1997), the clastogenic potential of sulfoxaflor (purity 96.6%) dissolved in DMSO was

tested in rat lymphocytes (whole blood). Based on the results of the mitotic index, concentrations of 693.3, 1386.5 and 2773 µg/ml were used with and without metabolic activation in an experiment with 4 hours of treatment and harvest at 24 hours (i.e. 20 hours post-exposure). In addition, with 24 hours of treatment without S9 mix, concentrations of 173.3, 346.6 and 693.3 µg/ml were chosen. Vehicle (DMSO) and positive controls (cyclophosphamide and mitomycin C for the test with and without metabolic activation, respectively) were included to demonstrate the sensitivity of the test system. Cultures were set up in duplicate. After slide preparation and staining of the cells, 200 metaphases per dose and treatment condition were analysed for chromosomal aberrations.

None of the cultures treated with sulfoxaflor in the absence or in the presence of S9 mix showed any biologically relevant or statistically significant increase in the numbers of aberrant metaphases. No change in the frequency of polyploid metaphases was observed. The positive controls mitomycin C and cyclophosphamide induced clastogenic effects and demonstrated the sensitivity of the test system and the activity of the S9 mix used. Based on the results of this test, sulfoxaflor was considered not to be clastogenic for mammalian cells in vitro, in either the presence or absence of metabolic activation (Schisler, Geter & Kleinert, 2007).

In a mammalian erythrocyte micronucleus test conducted according to OECD test guideline 474 (1997), groups of six male and six female Crl:CD1(ICR) mice received two oral (gavage) doses (24 hours apart) of sulfoxaflor (purity 95.6%) at 0 (0.5% aqueous hydroxypropyl methylcellulose/methylcellulose polymers), 100, 200 or 400 mg/kg bw in a volume of 10 ml/kg bw. The doses were selected based on the results of a range-finding experiment (250, 500, 1000, 1500, 2000 mg/kg bw per day, 3–4 animals of each sex). The vehicle served as negative control, and cyclophosphamide (one dose at 120 mg/kg bw) served as positive control. The animals were terminated 24 hours after the last administration, the bone marrow of the two femora was prepared and 2000 polychromatic erythrocytes were evaluated per animal (five animals) and investigated for micronuclei. The ratio of polychromatic erythrocytes to normochromatic erythrocytes was determined in 200 erythrocytes.

In the range-finding experiment, most of the animals (both sexes) died within 2 hours after administration of doses of 1000 mg/kg bw per day and above. Animals dosed with 250 or 500 mg/kg bw per day showed little or no clinical signs of toxicity.

In the main study, decreased activity was observed in sulfoxaflor-treated mice at 400 mg/kg bw. There was no substance-induced mortality. No signs were recorded for the control groups or other dose groups, and no animals died in these groups. Body temperature was decreased in animals dosed with 400 mg/kg bw per day.

Two oral doses of sulfoxaflor up to and including 400 mg/kg bw did not lead to any increase in the number of polychromatic erythrocytes with micronuclei. No inhibition of erythropoiesis, determined from the ratio of polychromatic to normochromatic erythrocytes, was observed. The positive control caused a clear increase in the number of polychromatic erythrocytes with micronuclei, thus demonstrating the sensitivity of the test system. Based on the results of this test, sulfoxaflor was considered not to be clastogenic in vivo in mice (LeBaron & Schisler, 2009).

2.5 Reproductive toxicity

(a) Multigeneration studies

In a reproduction/developmental toxicity screening study conducted according to OECD test guideline 421, groups of 12 male and 12 female Crl:CD(SD) rats were given diets containing sulfoxaflor (purity 95.6%) at a concentration of 0, 100, 500 or 1000 ppm (equal to 0, 8.26, 40.7 and 79.1 mg/kg bw per day in males and 0, 8.30, 42.2 and 81.6 mg/kg bw per day in females, respectively). Males were fed the test diets for 2 weeks prior to breeding and continuing throughout breeding until

termination. The females were fed the test diets for 2 weeks prior to breeding, continuing through breeding (up to 2 weeks), gestation, lactation and weaning; pups were weaned on postnatal day (PND) 21. Effects on gonadal function, mating behaviour, conception, development of the conceptus, parturition and postnatal growth and survival were evaluated. In addition, a gross necropsy and histopathological examination of the adults were conducted with an emphasis on organs of the reproductive system. In the offspring, litter size, pup survival, sex, body weight and the presence of gross external morphological alterations were assessed.

All parental animals survived until termination, and there were no treatment-related clinical observations at any dose level throughout the study. Males in the 1000 and 500 ppm dose groups had a treatment-related decrease in feed consumption and body weight (1000 ppm only) during the 1st week of treatment. Females in the 1000 and 500 ppm dose groups had treatment-related decreases in body weight gain during the 1st week of gestation, and females in the 1000 ppm dose group had slightly lower feed consumption during the pre-breeding and gestation phases.

Males of the 1000 and 500 ppm dose groups had increased absolute and relative liver weights that were dose and treatment related (liver weights for females at 1000 ppm were not recorded due to an effect on litter survival; there was no effect at 500 ppm). Treatment-related histological effects were observed in the livers of males given 1000 and 500 ppm and females given 1000 ppm and consisted of a dose-related increase in the severity of hepatocellular hypertrophy, with altered tinctorial properties, involving the centrilobular to midzonal regions of the hepatic lobule. Histological effects were of very slight or slight severity in males and very slight severity in females. In addition, there was treatment-related multifocal hepatocyte vacuolation (slight severity) in 1000 ppm females (Table 22).

There were no reproductive or developmental toxicity effects observed in any group up to PND 0 (birth). Shortly after birth, there was a significant decrease in pup survival in the 1000 ppm dose group, such that PND 1 survival was 46.3%, compared with 98.3% in controls. In addition, PND 1 pup body weights were significantly decreased (22–25%) relative to controls. By PND 4, 11 of 12 dams had total litter loss, resulting in 7.3% pup survival, compared with 95.4% in controls. Because only 1 of 12 litters remained, this dose group was terminated on PND 6. Pup survival was also affected in the 500 ppm group, with 4 of 12 dams losing approximately half of their litters by PND 4, which resulted in a pup survival rate of 81.2% compared with 95.4% in controls. There were no effects on pup body weight in the 500 ppm group. There were no treatment-related effects on any other reproductive parameters at 1000 or 500 ppm, and no reproductive effects whatsoever at 100 ppm (Table 23).

Analyses of plasma samples from PND 4 culled pups indicated a dose-proportionate increase in the systemic concentration of sulfoxaflor. In plasma and milk samples taken from three dams of the 1000 ppm group on PND 4 or PND 6, the concentrations of sulfoxaflor were equivalent (26.8 µg/g in plasma, 28.3 µg/g in milk).

The NOAEL for parental toxicity was 100 ppm (equal to 8.26 mg/kg bw per day), based on decreased body weight gains in females during the 1st week of gestation at 500 ppm (equal to 40.7 mg/kg bw per day) and above.

The NOAEL for effects on offspring was 100 ppm (equal to 8.26 mg/kg bw per day), based on reduced pup survival at 500 ppm (equal to 40.7 mg/kg bw per day) and above (Rasoulpour et al., 2010b).

In a two-generation reproductive toxicity study conducted according to OECD test guideline 416, groups of 27 male and 27 female Crl:CD(SD) rats were given diets containing sulfoxaflor (purity 95.6%) at a concentration of 0, 25, 100 or 400 ppm, equal to 0, 1.52–1.74, 6.07–6.86 and 24.6–28.1 mg/kg bw per day in males (F_0 and F_1 generation, respectively, for the entire dosing period) and 0, 1.91–2.11, 7.82–8.39 and 30.5–34.3 mg/kg bw per day in females (F_0 and F_1 generation, respectively, for the pre-mating period), for approximately 10 weeks prior to breeding and continuing through

Table 22. Summary of selected parental findings in a reproduction/developmental toxicity screening study in rats

	Males				Females			
	Dietary concentration (ppm)							
	0	100	500	1000	0	100	500	1000
Body weight (g), pre-mating								
- day 7	312.4	309.1	304.0	293.9*	212.5	206.3	205.8	202.1
- day 14	366.6	360.1	354.4	343.4*	239.1	225.9	225.9	226.0
Body weight gain (g), gestation								
- days 0–7	—	—	—	—	49.2	48.9	40.6*	37.0*
- days 0–20	—	—	—	—	162.1	171.0	151.5	150.7
Feed consumption (g/day), pre-mating								
- days 1–7	26.4	25.2	24.0*	22.2*	18.1	17.3	17.0	16.0
- days 7–14	27.5	25.9	25.7	24.8	19.3	17.0*	17.8	17.4*
Terminal body weight (g)	405.3	402.2	397.0	386.9	298.3	291.7	287.1	284.7
Liver weight, absolute (g)	12.80	12.66	14.08	14.55	12.34	12.18	12.43	12.74
Liver weight, relative (% of body weight)	3.14	3.14	3.54*	3.75*	4.13	4.18	4.33	4.47
Hepatocellular hypertrophy (no.)								
- very slight	0	0	10	0	1	0	0	12
- slight	0	0	1	12	0	0	0	0
Hepatocellular vacuolation (no.)								
- very slight	7	8	8	7	11	10	6	8
- slight	0	0	0	0	0	0	0	2

From Rasoulpour et al. (2010b)
* $P < 0.05$

Table 23. Summary of selected findings for reproductive performance and litter parameters in rats

	0 ppm	100 ppm	500 ppm	1000 ppm
Mating index (%)	100	100	100	100
Fertility index (%)	100	100	100	100
Gestation index (%)	100	100	100	100
Gestation length (days)	21.4	21.3	21.8	21.7
Time to mating (days)	2.5	2.8	3.4	3.3
Mean litter size (born live)				
- PND 0	14.4	14.9	13.8	14.8
- PND 1	14.2	14.9	13.0	6.8*
- PND 4 (before/after culling)	13.8/8.0	14.6/8.0	11.2/7.8	1.6*/1.0*
- PND 7	8.0	7.8	7.8	—
- PND 21	8.0	7.8	7.8	—
Survival index (%)				
- PND 1	98.3	100.0	94.5[a]	46.3*
- PND 4, before culling	95.4	97.8	81.2[a]	7.3*
- PND 21, after culling	100.0	97.9	100.0	—
Mean pup body weight (g), males/females				
- PND 1	6.8/6.3	6.6/6.4	6.7/6.3	5.1*/4.9*
- PND 4, before culling	9.5/9.0	9.0/8.5	9.3/8.8	7.1/7.3
Plasma concentration of sulfoxaflor (µg/g), PND 4	0	1.3	7.4	15.8

From Rasoulpour et al. (2010b)
* $P < 0.05$
[a] Outside historical control range (PND 1: 96.2–100%; PND 4: 94.0–100%).

breeding, gestation and lactation for two generations. In-life parameters included clinical observations, feed consumption, body weights, estrous cyclicity, reproductive performance, pup survival, pup body weights, puberty onset and anogenital distance. In addition, postmortem evaluations included gross pathology and organ weights in weanlings and toxicokinetic analyses, gross pathology, organ weights, oocyte quantification and sperm count, motility and morphology, and histopathology in adults.

In the parental animals, there were no treatment-related mortalities or clinical observations and no treatment-related effects on body weight or feed consumption in either generation at any dose level. Also, there were no treatment-related effects on estrous cyclicity, sperm analysis parameters, mating, conception, fertility or gestation indices, time to mating or gestation length in either generation at any dose level.

Parental postmortem observations consisted of increased absolute and relative liver weights in the F_0 (12.8% and 10.9%, respectively) and F_1 (6.5% and 7.8%, respectively) males at 400 ppm. This effect on liver weight correlated with histopathological findings of very slight to slight centrilobular hepatocyte hypertrophy, often with a very slight increase in individual cell necrosis of centrilobular hepatocytes (Table 24). No other systemic effects were noted at 400 ppm, and there were no treatment-related effects on F_0 or F_1 parameters in male or female rats at 25 or 100 ppm.

Reproductive effects were limited to the 400 ppm dose group and comprised slightly decreased neonatal (PNDs 1 and 4) survival in both generations. As a consequence of this effect on neonatal pup survival, there was a statistically significant decrease in gestation survival index (percentage of live born pups/total pups delivered) in the high-dose F_2 litters. As gestation survival index is a ratio of live pups over total pups delivered, this slight decrease in the high-dose group index is attributed to treatment-related neonatal deaths known to occur at or shortly after birth with high exposures to sulfoxaflor (Table 25).

Evidence indicating that survival is not affected before birth comes from a cross-fostering study, developmental toxicity study and two critical windows of exposure studies, all demonstrating that in utero exposure to sulfoxaflor caused postnatal, and not gestational, death. Given the clear profile of neonatal deaths, effectively coincident with birth and during the very early postnatal period, it is most appropriate to combine the gestation survival index data with PND 1–4 survival to create a combined "PND 0–4 survival" category. Here, there is a clear treatment-related, statistically significant decrease in total pup survival in the 400 ppm F_2 litters (Table 25).

In addition, there was an apparent treatment-related, statistically significant delay (2.4 days) in puberty onset (preputial separation) for F_2 males at 400 ppm without a corresponding decrement in body weight at the time of attainment (Table 25). This external marker of male puberty onset is androgen dependent, but the underlying mechanism to explain how sulfoxaflor induced this effect is not known; however, there were no other indications of androgenic or anti-androgenic effects. This included no treatment-related effects on anogenital distance, no effects on testis or accessory sex gland (i.e. prostate, seminal vesicle and epididymis) weight or histopathology, no evidence of malformations, such as hypospadias or ectopic testes, no effects on mating, fertility, time to mating or gestation length, and no treatment-related effects on preputial separation at the same dose level in a developmental neurotoxicity study with sulfoxaflor. Taken together, the weight of evidence across androgen-sensitive end-points does not support any other sulfoxaflor-mediated anti-androgenic effects. There were no effects on puberty onset or any other parameter of reproductive performance or offspring growth and survival at 25 or 100 ppm.

Owing to the statistically significant delay in male puberty onset at 400 ppm in the F_1 offspring, anogenital distance measurements on F_2 litters were triggered as specified in the relevant test guidelines. There were no treatment-related effects on absolute or relative anogenital distance in male or female pups at any dose level. Although female anogenital distance and relative anogenital distance were statistically significantly reduced at 25 and 400 ppm, these differences were not considered treatment related, as they did not exhibit a dose–response relationship (Table 25). Furthermore, anogenital

Table 24. Summary of selected parental findings in a two-generation reproductive toxicity study in rats

	Males				Females			
	Dietary concentration (ppm)							
	0	25	100	400	0	25	100	400
F_0 generation								
Body weight (g), pre-mating, day 71	523.8	543.7	529.5	540.1	303.4	295.1	298.4	290.2
Terminal body weight (g)	568.0	584.6	578.3	584.4	313.1	309.1	306.6	308.3
Liver weight, absolute (g)	15.09	15.98	15.49	17.02*	9.61	9.91	9.57	10.02
Liver weight, relative (% of body weight)	2.65	2.73	2.68	2.91*	3.06	3.20	3.12	3.26
Liver, no. examined histopathologically	*27*	*27*	*27*	*27*	*27*	*6*	*6*	*27*
Hepatocellular hypertrophy								
- very slight	0	0	0	2	0	0	0	0
- slight	0	0	0	24	0	0	0	0
Hepatocellular necrosis, single cell, centrilobular								
- very slight	9	7	9	25	3	0	0	5
Hepatocellular vacuolation/fatty change, centrilobular								
- very slight	0	0	2	4	0	0	0	0
- slight	1	0	0	2	0	0	0	0
F_1 generation								
Body weight (g), pre-mating, day 71	559.7	573.1	562.8	556.0	302.7	301.5	301.5	292.5
Terminal body weight (g)	630.3	640.0	633.4	624.5	322.0	323.1	320.6	309.9
Liver weight, absolute (g)	17.45	17.47	17.32	18.59	10.10	10.44	10.14	10.15
Liver weight, relative (% of body weight)	2.76	2.72	2.74	2.98*	3.14	3.23	3.17	3.27
Liver, no. examined histopathologically	*27*	*27*	*27*	*27*	*27*	*6*	*6*	*27*
Hepatocellular hypertrophy								
- very slight	0	0	1	19	0	0	0	0
- slight	0	0	0	7	0	0	0	0
Hepatocellular necrosis, single cell, centrilobular								
- very slight	6	6	4	12	1	0	0	0
Hepatocellular vacuolation/fatty change, centrilobular								
- very slight	1	0	0	0	0	0	0	0

From Rasoulpour et al. (2010c)
* $P < 0.05$

distance was decreased in treated females, relative to controls, whereas alterations in endocrine function would typically result in an increase in anogenital distance in females (i.e. masculinization).

Toxicokinetic data from lactation day (LD) 4 dams and culled PND 4 pups in the second generation show dose-proportional systemic exposure to sulfoxaflor in dams and their offspring. Plasma concentrations of sulfoxaflor in rat pups were, on average, 32% of the levels measured in the dams (Table 25).

The NOAEL for parental toxicity was 100 ppm (equal to 6.07 mg/kg bw per day), based on liver toxicity (increased incidence of vacuolation/fatty change of centrilobular hepatocytes) in F_0 males at 400 ppm (equal to 24.6 mg/kg bw per day).

Table 25. Summary of selected findings for reproductive performance and litter parameters in rats

	0 ppm	25 ppm	100 ppm	400 ppm
F_1 generation				
Mating index (%), males/females	92.6/92.6	100/100	100/100	100/100
Fertility index (%), males/females	92.6/92.6	100/96.3	88.9/88.9	100/100
Gestation index (%)	100	100	100	100
Gestation length (days)	21.7	21.5	21.6	21.6
Time to mating (days)	2.7	3.3	2.3	3.3
Gestation survival (%)	99.2	99.5	99.7	100
Postimplantation loss (%)	5.92	5.85	8.23	7.24
Mean litter size (born live)				
- PND 0	14.8	14.7	14.5	13.8
- PND 1	14.7	14.6	14.3	13.5
- PND 4	14.5	14.4	14.2	13.1
- PNDs 7, 14 and 21	8.0	8.0	7.8	7.7
PND 1 survival (%)	99.4	99.2	99.1	98.1
PND 4 survival (%)	97.2	97.9	97.1	95.4
F_2 generation				
Mating index (%), males/females	96.3/96.3	92.0/92.3	100/100	96.2/96.3
Fertility index (%), males/females	92.6/92.6	92.0/92.3	92.3/92.3	92.3/92.6
Gestation index (%)	100	100	100	100
Gestation length (days)	21.6	21.7	21.7	21.5
Time to mating (days)	3.0	2.7	2.3	2.6
Gestation survival (%)	99.7	99.1	98.8	97.4*
Postimplantation loss (%)	7.35	8.11	6.87	14.03[a]
Mean litter size (born live)				
- PND 0	13.2	14.3	14.3	13.4
- PND 1	13.1	14.2	14.1	12.9
- PND 4	13.0	14.0	13.9	12.8
- PNDs 14 and 21	7.9	7.9	8.0	7.8
PND 1 survival (%)	99.7	99.1	98.5	96.7
PND 4 survival (%)	98.8	98.0	97.1	95.5
PND 0–4 survival (%)	98.5	97.1	96.0	93.0*[b]
Anogenital distance (mm)/body weight (g)				
- males	3.76/7.3	3.68/7.4	3.64/7.2	3.55/6.9
- females	2.13/6.9	1.97*/7.0	2.01/6.8	1.97*/6.6
Days to vaginal opening/body weight (g)	31.6/117.2	31.7/116.4	31.4/114.0	32.1/112.2
Days to preputial separation/body weight (g)	44.6/253.6	46.4/265.8	44.5/250.3	47.0*[c]/272.8
Test material intake by dams (mg/kg bw per day), LD 4	0	2.1	8.5	29.2
Plasma concentration in dams (µg/g), LD 4	0	1.1	4.5	15.9
Plasma concentration in male pups (µg/g), LD 4	0	< 0.6 (LLQ)	1.4	5.3
Plasma concentration in female pups (µg/g), LD 4	0	< 0.6 (LLQ)	1.5	5.8

From Rasoulpour et al. (2010c)

LD, lactation day; LLQ, lowest level quantified; PND, postnatal day; * $P < 0.05$

[a] Outside historical control range for postimplantation loss (5.06–10.7%).

[b] Outside historical control range for PND 0–4 survival (93.4–98.4%).

[c] Outside historical control range for preputial separation (44.3–45.7 days).

The NOAEL for effects on fertility was 400 ppm (equal to 24.6 mg/kg bw per day), the highest dose tested.

The NOAEL for effects on offspring was 100 ppm (equal to 6.07 mg/kg bw per day), based on reduced pup survival and delayed preputial separation (puberty onset) in F_2 males at 400 ppm (equal to 24.6 mg/kg bw per day) (Rasoulpour et al., 2010c).

In a cross-fostering study conducted to assess whether the observed effects of sulfoxaflor on neonatal survival in rats resulted from in utero and/or lactational exposure, groups of 32 time-mated female Crl:CD(SD) rats were given diets containing sulfoxaflor (purity 95.6%) at a concentration of 0 or 1000 ppm for 2 weeks prior to mating through weaning on LD 21. As the control and treated females mated, they were subdivided into foster dams and donor dams. A caesarean section was performed on gestation day (GD) 21. At this time, one or more batches of two of the offspring of each sex from donor dams were immediately cross-fostered to a foster dam that had its own litter removed that day (i.e. on LD 0). After cross-fostering was complete, each control and sulfoxaflor-treated foster dam had mixed litters composed of two pups of each sex that originated from control donor dams (five litters) and two pups of each sex that originated from sulfoxaflor-treated donor dams (eight litters). This design controlled for litter of origin effects and enabled comparison of the survival of pups exposed to sulfoxaflor during gestation alone or during lactation alone with unexposed control pups and pups exposed during both gestation and lactation.

Dams given 1000 ppm sulfoxaflor had treatment-related effects on body weight, body weight gain and feed consumption consistent with effects seen at this dose level in the previous reproduction/developmental toxicity screening study. Time-weighted average doses for treated animals were 81.2, 74.5 and 59.5 mg/kg bw per day in the pre-mating, gestation and lactation periods, respectively. These corresponded to maternal sulfoxaflor blood concentrations of 23.0–29.3 (mean 27.0) µg/g plasma on GD 21 and 19.6–25.0 (mean 22.4) µg/g plasma on LD 0. The average measured concentration of sulfoxaflor in plasma from male/female pups on GD 21 and LD 0 from these dams was 24.8/24.8 and 25.3/25.9 µg/g plasma, respectively. Thus, fetal and pup plasma levels of sulfoxaflor were very similar to one another and very similar to dam plasma levels. The measured sulfoxaflor concentrations in milk from the same dams on LD 0 were approximately half the corresponding plasma levels and ranged from 12.3 to 14.0 (mean 13.3) µg/g milk.

All offspring from dams exposed to 1000 ppm sulfoxaflor prior to birth died by PND 4, irrespective of whether they were cross-fostered to control or treated foster dams (Table 26). Consistent with reduced viability, some offspring were cold to the touch, had bluish skin, were autolysed and cannibalized and had stomachs void of milk.

Conversely, there was no effect on neonatal survival for pups exposed to sulfoxaflor only after birth. Furthermore, PND 1 pup body weights were significantly decreased in prenatally exposed offspring.

In conclusion, these data demonstrate that the effect of sulfoxaflor on pup survival was due to in utero, not lactational, exposure (Rasoulpour & Zablotny, 2010a).

(b) Developmental toxicity

Rats

In a prenatal developmental toxicity range-finding study, groups of seven time-mated female Crl:CD(SD) rats were given diets containing sulfoxaflor (purity 95.6%) at a concentration of 0, 500, 1000, 1500 or 2000 ppm (equal to 0, 35.4, 68.0, 86.7 and 94.2 mg/kg bw per day) on GD 6 through GD 21. In-life parameters evaluated for all groups included clinical observations, body weight, body weight gain and feed consumption. On GD 21, all surviving rats were euthanized and examined for gross pathological alterations. Liver and kidney weights were recorded, along with the number of corpora lutea, implantations, resorptions and live/dead fetuses.

Table 26. Summary of selected findings in a cross-fostering study in rats.

	Dietary concentration (ppm), in gestation/in lactation			
	0/0	1000/0	0/1000	1000/1000
Litter size				
- PND 0	4.0	4.0	4.0	4.0
- PND 1	4.0	0.6	3.9	1.6
- PND 4	4.0	0.0	3.9	0.0
Survival (%)				
- PND 1	100.0	15.0	96.9	40.6
- PND 4	100.0	0.0	96.9	0.0
Pup body weights (g), males/females				
- PND 0	5.5/5.2	5.2/5.1	5.7/5.3	5.5/5.1
- PND 1	6.0/5.5	4.5[a]/4.8	6.3/5.8	4.8/4.6

From Rasoulpour & Zablotny (2010a)
PND, postnatal day
[a] Only one litter with pups remaining on PND 1.

Administration of sulfoxaflor to time-mated rats resulted in excessive systemic toxicity at 1500 and 2000 ppm, as evidenced by body weight loss, decreased body weight gain and decreased feed consumption. Therefore, all animals in these groups were euthanized for humane reasons on GD 13, with no further collection of data. Animals in the 1000 ppm dose group had transient and less severe treatment-related decreases in body weight/body weight gain, decreased feed consumption and increased relative liver weights. The 500 ppm dose group had treatment-related, transient decreases in feed consumption during the first 3 days of treatment; however, the body weights and body weight gains remained comparable to those of controls. There were no treatment-related clinical observations in any group tested and no treatment-related gross pathology observations, effects on pregnancy rates, effects on numbers of corpora lutea and implantations, increase in resorption rate or litter size or indication of embryo/fetal lethality in animals given diets containing 500 or 1000 ppm sulfoxaflor (Rasoulpour, Marshall & Yano, 2008).

Based on the results of this range-finding study, dietary concentrations of 25, 150 and 1000 ppm sulfoxaflor were selected for the definitive developmental toxicity study.

In a prenatal developmental toxicity study conducted according to OECD test guideline 414, groups of 26 time-mated female Crl:CD(SD) rats were given diets containing sulfoxaflor (purity 95.6%) at a concentration of 0, 25, 150 or 1000 ppm (equal to 0, 1.95, 11.5 and 70.2 mg/kg bw per day) on GD 6 through GD 21. In-life maternal study parameters included clinical observations, body weight, body weight gain and feed consumption. On GD 21, all rats were euthanized, and all dams and fetuses were examined for gross pathological alterations. In addition, blood was collected from dams and fetuses to determine blood levels of the test material. Liver, kidneys and gravid uterine weights were recorded, along with the numbers of corpora lutea, uterine implantations, resorptions and live/dead fetuses. All fetuses were weighed, sexed and examined for external alterations. Approximately one half of the fetuses were examined for visceral and craniofacial alterations, whereas skeletal examinations were conducted on the remaining fetuses.

Maternal toxicity was evidenced at 1000 ppm by decreases in body weight and body weight gains, relative to controls, with concomitant decreased feed consumption, throughout the treatment period and increased relative liver weights (Table 27).

Table 27. Summary of selected findings in a prenatal developmental toxicity study in rats

	0 ppm	25 ppm	150 ppm	1000 ppm
Dose in dams (mg/kg bw per day), last week	0	1.6	9.3	64.2
Plasma concentration in dams (µg/g), GD 21	0	0.84	4.94	35.2
Plasma concentration in fetuses (µg/g), GD 21	0	0.64	4.07	30.0
No. of females pregnant	24	23	25	25
Body weight gain (g), GDs 6–9	15.5	15.7	17.3	0.8*
Body weight gain (g), GDs 6–21	152.9	150.1	154.0	118.6*
Body weight gain (g), GDs 0–21	189.4	187.8	192.5	152.4*
Terminal body weight (g), corrected	319.0	317.9	319.9	294.9*
Liver weight, absolute (g)	14.7	14.4	14.6	14.2
Liver weight, relative (% of body weight)	3.45	3.40	3.43	3.66*
Feed consumption (g/day), GDs 6–7	22.5	22.8	21.7	15.7*
Feed consumption (g/day), GDs 7–8	22.5	22.8	22.8	13.7*
Feed consumption (g/day), GDs 8–9	23.5	22.8	23.6	17.7*
No. of litters with viable fetuses	24	23	25	25
Number of corpora lutea per dam	14.1	14.1	14.3	13.5
Number of implantations per dam	13.5	13.3	13.9	13.0
Number of resorptions per litter	0.2	0.7	0.6	0.7
Mean postimplantation loss (%)	1.4	4.9	5.1	5.2
No. of live fetuses per litter	13.3	12.7	13.3	12.3*
Fetal body weights (g), males	5.94	6.02	6.02	5.29*
Fetal body weights (g), females	5.67	5.71	5.63	4.99*
Gravid uterine weight (g)	106.4	102.8	106.6	92.3*
External examination				
No. of fetuses/litters	320/24	278/22	332/25	295/24
Forelimb flexure, slight (no. of fetuses/litters)	0/0	0/0	0/0	154*/20[a]
Forelimb flexure (no. of fetuses/litters)	0/0	0/0	0/0	122*/23
Hindlimb rotation (no. of fetuses/litters)	0/0	0/0	0/0	12*/7
Visceral examination				
No. of fetuses/litters	168/24	139/22	173/26	149/24
Convoluted ureter (no. of fetuses/litters)	0/0	1/1	0/0	19*/7
Skeletal examination				
No. of fetuses/litters	152/24	126/22	159/25	133/24
Delayed ossification, parietal (no. of fetuses/litters)	0/0	0/0	1/1	5*/4
Bent clavicle (no. of fetuses/litters)	0/0	0/0	0/0	40*/17
Fused sternebrae (no. of fetuses/litters)	0/0	0/0	1/1	6*/5

From Rasoulpour, Marshall & Saghir (2010)
* $P < 0.05$
[a] Four litters were excluded.

Developmental toxicity was evidenced at 1000 ppm by decreases in fetal body weight and gravid uterine weight and clear increases in several fetal abnormalities (see below). The external examination at 1000 ppm revealed that 40% of the fetuses (122/295) had unilateral or bilateral forelimb flexure and 12 fetuses had hindlimb rotation abnormalities, whereas approximately 60%

of the fetuses (154/248) had unilateral or bilateral slight forelimb flexure (variation). The incidences of severe forelimb flexure, hindlimb rotation and slight forelimb flexure were statistically significant and considered treatment related. Fetuses in this group exhibited a contracted or hunched posture of the body, limbs and neck. This did not appear to be a structural defect, but instead was noted during visceral examination as a difficulty in laying the fetuses flat due to skeletal muscle contracture.

At the visceral examination at 1000 ppm, 19 of 149 fetuses had unilateral or bilateral convoluted ureter, the incidence of which reached statistical significance and was deemed treatment related. Two of these fetuses also had hydroureter.

The skeletal examination at 1000 ppm showed that there were 40 of 133 fetuses with unilateral or bilateral bent clavicle, which co-occurred with limb abnormalities in 35 of 40 fetuses. This finding was statistically significant and considered treatment related. Also at 1000 ppm, there was a treatment-related increase in the incidence of a skeletal variation, fused sternebrae (6/133 fetuses).

Administration of 150 or 25 ppm sulfoxaflor produced no treatment-related maternal toxicity and no indications of embryo/fetal toxicity or teratogenicity.

The terminal plasma concentrations of sulfoxaflor in both dam and fetal blood were dose proportional throughout the entire range of dietary exposure concentrations, with similar levels in the maternal and fetal blood compartments.

The NOAEL for maternal toxicity was 150 ppm (equal to 11.5 mg/kg bw per day), based on decreases in body weight and body weight gain and decreased feed consumption at 1000 ppm (equal to 70.2 mg/kg bw per day).

The NOAEL for prenatal developmental toxicity was 150 ppm (equal to 11.5 mg/kg bw per day), based on increases in several fetal abnormalities (forelimb flexure, bent clavicle, hindlimb rotation, fused sternebrae, convoluted ureter and hydroureter) at 1000 ppm (equal to 70.2 mg/kg bw per day) (Rasoulpour, Marshall & Saghir, 2010).

A special developmental toxicity study was conducted to determine the critical window of susceptibility of rat fetuses and to test the hypothesis that late gestational exposure to sulfoxaflor induces fetal abnormalities and reduced neonatal survival via its pharmacological action on the fetal muscle nAChR. This receptor develops functional expression between GD 16 and GD 17 in the rat, resulting in synchronized fetal limb movements and diaphragmatic responsiveness important for the transition to extrauterine respiration.

Groups of 12 time-mated female Crl:CD(SD) rats were given diets containing sulfoxaflor (purity 95.6%) at a concentration of 0 or 1000 ppm beginning on GD 6 or 16. Group 1 received control feed (0 ppm) from GD 6 until termination on LD 4. Group 2 was administered feed containing 1000 ppm sulfoxaflor from GD 6 until the morning of GD 16, to cover all of embryogenesis up to, but not including, the start of early fetal movements, and was then switched to control feed (0 ppm) until termination on LD 4. Group 3 was administered control feed (0 ppm) from GD 6 until the morning of GD 16, offered feed containing 1000 ppm sulfoxaflor from GD 16 until parturition in order to cover development of the muscle nAChR and its role in development of synchronized fetal limb movements up to onset of parturition and switched back to control feed (0 ppm) until termination on LD 4. For toxicokinetic analysis, a blood sample was taken from four females per group on GD 16 (control and group 2) and GD 21 (control and group 3) to measure plasma sulfoxaflor concentration.

In the offspring, effects on litter size, survival, body weight and the presence of gross external morphological alterations, with particular focus on limb abnormalities (e.g. forelimb flexure and hindlimb rotation), were carefully assessed. All pups surviving to PND 4 were examined for gross external alterations and euthanized by oral administration of sodium pentobarbital solution. Following external examination, one randomly selected pup of each sex from groups 1 and 2 was examined internally for convoluted ureters. In addition, one randomly selected pup or all group 3 pups of each

sex per litter were viscerally examined for convoluted ureters. Following visceral examination, the pups were preserved in alcohol, macerated, stained with Alizarin Red-S in order to visualize ossified bone, cleared and examined for bent clavicles. Any pups found dead or that were euthanized in moribund condition were examined to the extent possible and discarded.

Offspring from animals given 1000 ppm sulfoxaflor from GD 6 to GD 16 (group 2) were completely normal and did not display previously described fetal abnormalities or reduced neonatal survival. In contrast, offspring given 1000 ppm sulfoxaflor from GD 16 to birth (group 3) had the same gross effects of limb contractures and reduced neonatal survival seen in the previous studies that had treatment with 1000 ppm sulfoxaflor throughout gestation (Table 28). This demonstrates that the critical window of susceptibility for both of these effects falls within GD 16 to birth.

In addition, daily examination of group 3 offspring born with limb abnormalities confirmed that these were reversible in a number of pups from five litters shortly after withdrawal of maternal dietary exposure to sulfoxaflor (Table 29). In some cases, full reversal of the limb abnormalities was evident the day after birth and occurred for all affected animals that survived to PND 4; reversal also occurred in some animals that subsequently died before PND 4. Likewise, the visceral and skeletal findings of abnormal ureter and bent clavicle, the latter of which had a high incidence (30.1% of fetuses), in the definitive developmental toxicity study were not present in this study at necropsy on PND 4, despite similar blood concentrations and limb abnormality indices between these two studies.

The study demonstrated that the critical period of developmental susceptibility to sulfoxaflor-induced fetal abnormalities and reduced neonatal survival is between GD 16 and birth and that the fetal limb abnormalities were rapidly reversible after birth in pups surviving to PND 4. These results support the hypothesis that late gestational exposure to sulfoxaflor induces fetal abnormalities and neonatal death via its pharmacological action on the fetal muscle nAChR, which develops functional expression during this stage of gestation (Rasoulpour & Zablotny, 2010b).

In a subsequent special developmental toxicity study conducted to determine the critical window of susceptibility of rat fetuses, the GD 16 to birth exposure period was divided into three 48-hour exposure windows starting on the morning of GD 16, 18 or 20. Groups of 10 female Crl:CD(SD) rats were given control diet (group 1) or diets containing 1000 ppm sulfoxaflor (purity 95.6%) from GDs 16 to 18 (group 2), GDs 18 to 20 (group 3) or GDs 20 to 22 (group 4). At the end of their respective 48-hour treatment periods, dams were switched to control feed (0 ppm) until termination on LD 4. For toxicokinetic analysis, blood samples were taken from dams in the treated groups at the end of their 48-hour treatment interval.

In the offspring, effects on litter size, survival, body weight and the presence of gross external morphological alterations, with particular focus on limb abnormalities (e.g. forelimb flexure and hindlimb rotation), were carefully assessed. All pups surviving to PND 4 were examined for gross external alterations and euthanized by oral administration of sodium pentobarbital solution. Following external examination, all pups of each sex from groups 1 and 4 were viscerally examined for convoluted ureters. Following visceral examination, the pups were preserved in alcohol, macerated, stained with Alizarin Red-S in order to visualize ossified bone, cleared and examined for bent clavicles. All pups from groups 2 and 3 were discarded following examination for gross external alterations. Any pups found dead or that were euthanized in moribund condition were examined to the extent possible and discarded.

Offspring from animals given 1000 ppm sulfoxaflor for 48 hours starting on the morning of GD 16 or 18 (groups 2 and 3) were similar to controls and did not display previously described fetal abnormalities or reduced neonatal survival. In contrast, offspring from animals given 1000 ppm sulfoxaflor for 48 hours starting on the morning of GD 20 (group 4) had fetal limb abnormalities (forelimb flexure and hindlimb rotation) as well as reduced neonatal survival, demonstrating that exposure shortly before birth (GD 21 or 22) is sufficient to induce developmental toxicity (Table 30).

Table 28. Summary of selected findings in a special developmental toxicity study in rats

	0 ppm	1000 ppm	1000 ppm
	Group 1	Group 2	Group 3
Treatment period	Control	GDs 6–16	GD 16–birth
Dose (mg/kg bw per day)			
- GDs 6–16	0	76.5 (65.7–86.1)	0
- GDs 16–21	0	0	38.6 (20.8–53.8)
Plasma concentration in dams (µg/g)			
- GD 16	0	35.4–40.9	—
- GD 21	0	—	32.1–43.2
Body weight gain (g)			
- GDs 6–9	14.1	−2.0*	12.4
- GDs 6–16	75.1	50.2*	67.6
- GDs 16–17	10.6	17.1*	5.6
- GDs 19–21	29.4	23.5	17.2*
- GDs 16–21	70.6	65.6	43.4*
- GDs 0–21	181.4	143.9*	142.9*
Feed consumption (g/animal per day)			
- GDs 6–7	22.6	16.3*	22.7
- GDs 7–8	24.4	13.4*	23.7
- GDs 19–20	23.8	20.7*	13.7*
- GDs 20–21	23.8	19.8	15.5*
- LDs 1–4	36.0	31.1	25.5*
Litter size			
- born live	13.6	12.0	11.9
- born dead	0.4	0.2	0.3
- PND 1	13.6	12.0	9.9
- PND 2	13.6	12.0	5.9*
- PND 3	13.4	12.0	5.7*
- PND 4	13.3	11.9	5.6*
Pup survival (%)			
- PND 1	100.0	100.0	83.2*
- PND 2	100.0	100.0	49.7*
- PND 3	99.20	100.0	47.6*
- PND 4	98.4	100.0	46.9*
Pup body weight (g), males/females			
- PND 1	7.2/6.8	7.3/7.0	5.7*/5.5*
- PND 4	10.6/10.1	10.5/10.3	8.6*/8.1*
External examination of pups			
PND 0			
No. of fetuses/litters examined	122/9	120/10	143/12
Forelimb flexure (no. of fetuses/litters)	0/0	0/0	50/11*
Hindlimb rotation (no. of fetuses/litters)	0/0	0/0	19/8*

Table 28 (continued)

	0 ppm	1000 ppm	1000 ppm
	Group 1	Group 2	Group 3
PND 1			
No. of fetuses/litters examined	122/9	120/10	119/12
Forelimb flexure (no. of fetuses/litters)	0/0	0/0	38/9*
Hindlimb rotation (no. of fetuses/litters)	0/0	0/0	8/6*
PND 2			
No. of fetuses/litters examined	122/9	120/10	71/8
Forelimb flexure (no. of fetuses/litters)	0/0	0/0	6/4*
Hindlimb rotation (no. of fetuses/litters)	0/0	0/0	2/2
PND 4			
No. of fetuses/litters examined	122/9	120/10	67/7
Forelimb flexure (no. of fetuses/litters)	0/0	0/0	0/0
Hindlimb rotation (no. of fetuses/litters)	0/0	0/0	0/0

From Rasoulpour & Zablotny (2010b)
GD, gestation day; LD, lactation day; PND, postnatal day; * $P < 0.05$

Table 29. Summary of limb alteration reversals in rat pups from group 3 (treatment period GD 16 to birth)

Day		Litter No.											
		708	709	710	711	712	713	714	715	716	717	718	719
LD 0	No. of pups alive	11	12	1	15	11	12	14	12	14	15	12	14
	No. w/LABN	8	4	0	6	2	6	6	9	5	3	3	3
	No. born dead	1	0	0	0	0	0	0	1	0	1	0	0
LD 1	No. of pups alive	7	11	1	13	3	5	14	12	14	15	12	12
	No. w/LABN	7	3	0	4	0	5	2	5	7	1	2	6
	No. dead	4	1	0	2	8	7	0	0	0	0	0	2
	No. of reversals	—	—	—	—	—	—	4	4	—	2	1	—
LD 2	No. of pups alive	0	0	0	10	3	1	14	0	12	15	12	4
	No. w/LABN	LL	LL	LL	2	0	1	1	LL	2	1	0	1
	No. dead	11	12	1	5	8	11	0	12	2	0	0	10
	No. of reversals	—	—	—	—	—	—	1	—	3	—	2	—
LD 3	No. of pups alive	0	0	0	9	3	0	14	0	12	14	12	4
	No. w/LABN	LL	LL	LL	0	0	LL	0	LL	0	1	0	0
	No. dead	11	12	1	6	8	12	0	12	2	1	0	10
	No. of reversals	—	—	—	—	—	—	1	—	2	—	—	—
LD 4	No. of pups alive	0	0	0	9	3	0	14	0	12	14	12	3
	No. w/LABN	LL	LL	LL	0	0	LL	0	LL	0	0	0	0
	No. dead	11	12	1	6	8	12	0	12	2	1	0	11
	No. of reversals	—	—	—	—	—	—	—	—	—	1	—	—
No. w/LABN per litter		8	4	0	6	2	6	6	9	7	3	3	6
No. dead per litter		11	12	1	6	8	12	0	12	2	1	0	11
No. of confirmed reversals		—	—	—	—	—	—	6	4	5	3	3	—

From Rasoulpour & Zablotny (2010b)
LD, lactation day; LL, lost litter; w/LABN, with limb abnormalities (forelimb flexure and hindlimb rotation)

Table 30. Summary of selected findings in a special developmental toxicity study in rats

	0 ppm	1000 ppm	1000 ppm	1000 ppm
	Group 1	Group 2	Group 3	Group 4
Treatment period	Control	GDs 16–18	GDs 18–20	GDs 20–22
Dose (mg/kg bw per day)	0	63.9 ± 4.5	42.5 ± 1.3	35.7 ± 9.4
Plasma concentration in dams (µg/g)				
- GD 18, 20 or 22	0	16.4–33.3	23.0–30.2	16.1
- LD 0	0	—	—	5.4–6.7
Body weight gain (g)				
- GDs 16–17	3.6	−4.1*	4.1	3.8
- GDs 18–19	9.3	14.7	0.1*	8.4
- GDs 19–20	12.6	14.1	5.4*	14.3
- GDs 20–21	8.4	12.1	15.5	3.1
Feed consumption (g/animal per day)				
- GDs 16–17	25.1	21.1*	26.9	26.5
- GDs 18–19	18.9	18.6	14.3*	19.0
- GDs 19–20	21.1	22.6	14.2*	21.9
- GDs 20–21	19.6	21.5	20.1	14.4*
- GDs 21–22	11.7	13.1	13.3	2.9*
Litter size				
- born live	11.4	11.0	11.7	12.0
- born dead	0	0.1	0	0
- PND 1	11.4	10.9	11.6	10.8
- PND 2	11.4	10.9	11.6	10.8
- PND 3	11.4	10.9	11.6	10.8
- PND 4	11.3	10.9	11.6	10.8
Pup survival (%)				
- PND 1	100	99.0	99.0	99.0
- PND 2	100	99.0	99.0	89.6
- PND 3	100	99.0	99.0	89.6
- PND 4	99.1	99.0	99.0	89.6
External examination of pups				
PND 0				
No. of fetuses/litters examined	114/10	99/9	105/9	96/8
Forelimb flexure (no. of fetuses/litters)	0/0	0/0	0/0	7/4*
Hindlimb rotation (no. of fetuses/litters)	0/0	0/0	0/0	11/6*
PND 1				
No. of fetuses/litters examined	114/10	98/9	104/9	95/8
Forelimb flexure (no. of fetuses/litters)	0/0	0/0	0/0	4/3
Hindlimb rotation (no. of fetuses/litters)	0/0	0/0	0/0	5/2
PND 2				
No. of fetuses/litters examined	114/10	98/9	104/9	86/8
Forelimb flexure (no. of fetuses/litters)	0/0	0/0	0/0	0/0
Hindlimb rotation (no. of fetuses/litters)	0/0	0/0	0/0	0/0
PND 4				
No. of fetuses/litters examined	113/10	98/9	104/9	86/8
Forelimb flexure (no. of fetuses/litters)	0/0	0/0	0/0	0/0
Hindlimb rotation (no. of fetuses/litters)	0/0	0/0	0/0	0/0

From Rasoulpour & Zablotny (2010c)
* $P < 0.05$

The sulfoxaflor plasma concentration of groups 2, 3 and 4 ranged from 16.4 to 33.3, 23.0 to 30.2 and 5.4 to 16.1 µg/g plasma, respectively (Table 30). The plasma concentration of sulfoxaflor in group 4 was lower than in the other groups because three of the four sampled rats had undergone parturition; therefore, feed consumption, and corresponding test material intake, in these animals was minimal. The sulfoxaflor plasma concentration from the one group 4 animal that had not given birth was 16.1 µg/g.

The daily examination of surviving group 4 offspring born with limb abnormalities indicated that these limb abnormalities were reversible in a number of surviving pups from six litters shortly after withdrawal of maternal dietary exposure to sulfoxaflor (Table 31). In some cases, full reversal of the limb abnormalities was evident the day after birth and occurred for all affected animals that survived to PND 4; reversal also occurred in some animals that subsequently died before PND 4. Likewise, the visceral and skeletal findings of abnormal ureter and bent clavicle, the latter of which had a high incidence (30.1% of fetuses), in the definitive developmental toxicity study were not present in this study at necropsy on PND 4.

This study demonstrated that the critical period of developmental susceptibility to sulfoxaflor-induced fetal abnormalities and reduced neonatal survival effects occurs shortly before birth and that the fetal limb abnormalities are rapidly reversible after birth. These results support the hypothesis that late gestational exposure to sulfoxaflor induces fetal abnormalities and neonatal death via its pharmacological action on the fetal muscle nAChR, which develops functional expression during this stage of gestation (Rasoulpour & Zablotny, 2010c).

Rabbits

In a prenatal developmental toxicity range-finding study, groups of seven time-mated female New Zealand White rabbits received sulfoxaflor (purity 95.6%) by oral gavage at a dose level of 0, 10, 15, 20 or 25 mg/kg bw per day from GD 7 to GD 27. The test substance was administered as an aqueous suspension in 0.5% methylcellulose at a dose volume of 2 ml/kg bw. In-life parameters evaluated for all groups included clinical observations, body weight, body weight gain and feed consumption. On GD 28, all surviving rabbits were euthanized and examined for gross pathological alterations. Liver and kidney weights were recorded, along with the number of corpora lutea, implantations, resorptions and live/dead fetuses. Blood samples from five rabbits from each surviving dose group were taken at 1, 2, 4, 8 and 24 hours after the final dose on GD 27 for analysis of sulfoxaflor levels.

Oral administration of sulfoxaflor by gavage to time-mated New Zealand White rabbits at 25 or 20 mg/kg bw per day caused severe inanition, and the animals were removed from study on GD 13 or 16, respectively. Animals in the 15 mg/kg bw per day group had treatment-related body weight loss (14–78 g) upon initiation of dosing (GDs 7–10) and an overall decreased mean body weight gain (approximately 39% lower than controls) throughout the dosing period (GDs 7–28). There was no maternal toxicity observed at 10 mg/kg bw per day. There was no indication of embryo/fetal lethality at any dose level.

Toxicokinetic analyses on GDs 27–28 indicated slow elimination of sulfoxaflor from plasma, with a half-life of 14 hours. A 1.5-fold increase in the dose (from 10 to 15 mg/kg bw per day) resulted in a 1.4-fold increase in the daily systemic dose ($AUC_{24\ h}$: 236 and 332 µg·h/ml, respectively). The maximum concentrations (C_{max}) in plasma were observed at 2 hours after administration, with levels of 14.9 or 21.8 µg/g at 10 or 15 mg/kg bw per day, respectively (Rasoulpour & Brooks, 2008).

In a prenatal developmental toxicity range-finding study, groups of five time-mated female New Zealand White rabbits were given diets containing sulfoxaflor (purity 95.6%) at a concentration of 0, 500 or 1000 ppm (equal to 0, 21.7 and 36.6 mg/kg bw per day) on GD 7 through GD 28. In-life parameters evaluated for all groups included clinical observations, body weight, body weight gain and feed consumption. In addition, blood was collected from all surviving rabbits at 1, 2, 4 and

Table 31. Summary of limb alteration reversals in rat pups from group 4 (treatment period GDs 20–22)

Day		Litter No.							
		1636	1637	1639	1640	1642	1643	1644	1645
LD 0	No. of pups alive	12	11	14	14	12	12	9	12
	No. w/LABN	2	1	0	1	1	3	0	5
	No. born dead	0	0	0	0	0	0	0	0
LD 1	No. of pups alive	12	11	14	14	12	11	9	12
	No. w/LABN	2	0	0	0	0	1	0	4
	No. dead	0	0	0	0	0	1	0	0
	No. of reversals	—	1	—	1	1	1	—	1
LD 2	No. of pups alive	12	11	14	14	12	10	9	4
	No. w/LABN	0	0	0	0	0	0	0	0
	No. dead	0	0	0	0	0	2	0	8
	No. of reversals	2	—	—	—	—	—	—	—
LD 3	No. of pups alive	12	11	14	14	12	10	9	4
	No. w/LABN	0	0	0	0	0	0	0	0
	No. dead	0	0	0	0	0	2	0	8
	No. of reversals	—	—	—	—	—	—	—	—
LD 4	No. of pups alive	12	11	14	14	12	10	9	4
	No. w/LABN	0	0	0	0	0	0	0	0
	No. dead	0	0	0	0	0	2	0	8
	No. of reversals	—	—	—	—	—	—	—	—
No. w/LABN per litter		2	1	0	1	1	3	0	5
No. dead per litter		0	0	0	0	0	2	0	8
No. of confirmed reversals		2	1	—	1	1	1	—	1

From Rasoulpour & Zablotny (2010c)
LD, lactation day; w/LABN, with limb abnormalities (forelimb flexure and hindlimb rotation)

8 hours (GD 27) and 24 hours (GD 28) after the offering of feed on GD 27 to determine blood levels of test material. On GD 28, all surviving rabbits were euthanized and examined for gross abnormalities. Liver and kidney weights were recorded, along with the numbers of corpora lutea, implantations, resorptions and live/dead fetuses.

Treatment-related effects at 1000 ppm consisted of a statistically significant mean body weight loss of 60 g (range +19 to −173 g) after initiation of treatment (GDs 7–10) and a 33% decrease in mean body weight gain, relative to controls, throughout the treatment period (GDs 7–28). One rabbit in this group had 6 consecutive days of inanition (GDs 9–14) and was euthanized for humane reasons on GD 14. There was no maternal toxicity observed in the 500 ppm rabbits, and there was no indication of embryo/fetal lethality at any dose level.

Toxicokinetic analysis of the time-course plasma concentration of sulfoxaflor from the rabbits exposed through the diet showed that the daily systemic dose ($AUC_{24\ h}$: 439 and 776 µg·h/ml at 500 and 1000 ppm, respectively) was dose proportional, with constant steady-state plasma concentrations (18.3–19.4 and 32.8–35.6 µg/g at 500 and 1000 ppm, respectively) with minimal diurnal fluctuation, compared with the 3-fold difference between C_{min} and C_{max} observed after oral gavage. Dietary administration resulted in a dose-corrected $AUC_{24\ h}$ of about 22 µg·h/ml per milligram per kilogram body weight administered, which was consistent with previously reported dose-corrected values of

20–22 µg·h/ml per milligram per kilogram body weight administered following gavage administration. The dietary route afforded a greater applied maximally tolerated dose (1000 ppm, equal to 36.6 mg/kg bw per day) relative to gavage (15 mg/kg bw per day caused excessive maternal toxicity). Therefore, the dietary route of administration was chosen for the definitive rabbit developmental toxicity study, as it allows for more than twice the applied dose and a correspondingly higher $AUC_{24\,h}$ compared with gavage administration (Rasoulpour & Brooks, 2009a).

In a prenatal developmental toxicity study conducted according to OECD test guideline 414, groups of 26 time-mated female New Zealand White rabbits were given diets containing sulfoxaflor (purity 95.6%) at a concentration of 0, 30, 150 or 750 ppm (equal to 0, 1.3, 6.6 and 31.9 mg/kg bw per day) on GDs 7 through 28. In-life parameters evaluated for all rabbits included clinical observations, body weight, body weight gain and feed consumption. Maternal blood was collected for sulfoxaflor analysis from four rabbits per group over a 24-hour period starting on the morning of GD 27 and also at termination on GD 28. Fetal umbilical cord blood was also taken at termination. All rabbits surviving to GD 28 were euthanized and examined for gross pathological alterations and changes in liver, kidney and gravid uterine weight. The numbers of corpora lutea, uterine implantations, resorptions and live/dead fetuses were determined. All fetuses were weighed, sexed and examined for external and visceral alterations. Also, the heads were examined for craniofacial alterations by serial sectioning in approximately one half of the fetuses in each litter, whereas skeletal examinations were performed on all fetuses.

Animals in the 750 ppm dose group exhibited treatment-related maternal toxicity in the form of decreased faeces in 7 of 26 animals, decreased mean body weight gain (55%) from GD 7 to GD 13, decreased mean body weight gain (12%) throughout treatment (GDs 7–28) and decreased mean feed consumption (8–21%) from GD 7 to GD 17. There was no treatment-related maternal toxicity for animals in the 30 or 150 ppm dose groups. There was no treatment-related developmental toxicity in any dose group (Table 32).

The daily systemic dose of sulfoxaflor on GDs 27–28 was dose proportional, as indicated by the nearly identical mean dose-corrected $AUC_{24\,h}$ values of 18, 19 and 19 µg·h/ml per milligram per kilogram body weight administered for animals given 30, 150 and 750 ppm, respectively. Levels of sulfoxaflor in maternal and fetal blood were similar (Table 32). The daily systemic dose in this dietary study was similar to that measured in prior gavage studies with sulfoxaflor.

The NOAEL for maternal toxicity was 150 ppm (equal to 6.6 mg/kg bw per day), based on decreased faeces and decreases in body weight gain and feed consumption at 750 ppm (equal to 31.9 mg/kg bw per day).

The NOAEL for prenatal developmental toxicity was 750 ppm (equal to 31.9 mg/kg bw per day), the highest dose tested (Rasoulpour & Brooks, 2009b).

In a special developmental toxicity study conducted to assess the effects of sulfoxaflor on neonatal survival in rabbits, groups of 12 time-mated female New Zealand White (Hra:(NZW) SPF) rabbits that had two previous litters were given diets containing sulfoxaflor (purity 95.6%) at a concentration of 0 or 750 ppm (equal to 0 and 29 mg/kg bw per day) from GD 7 through the initiation of parturition (i.e. 25–26 consecutive days). All diets were provided at 150 g/day during the exposure period (GD 7 through initiation of parturition) and at 200 g/day during LDs 1–4; the control diet was offered to both groups after parturition. The F_0 females were approximately 9–13 months of age at the initiation of test substance exposure. All animals were observed twice daily for mortality and moribundity. Clinical observations, body weights and feed consumption were recorded at appropriate intervals. All F_0 females were allowed to deliver and rear their offspring to LD 4. All F_0 females were necropsied within 24 hours of total litter loss, on LD 4 or on post-mating day 37. All surviving F_1 offspring received a detailed physical examination on PND 4 and were then discarded.

Table 32. Summary of selected findings in a prenatal developmental toxicity study in rabbits

	0 ppm	30 ppm	150 ppm	750 ppm
No. of females pregnant	25	26	25	25
Body weight gain (g)				
- GDs 7–10	28.3	26.5	22.7	9.9
- GDs 10–13	74.8	72.2	73.7	41.9*
- GDs 7–28	356	340	348	315
Terminal body weight (g)	3502	3422	3463	3442
Feed consumption (g/day)				
- GDs 7–8	153	150	154	131*
- GDs 9–10	155	151*	150	142*
- GDs 12–13	150	144	141	122*
Gravid uterine weight (g)	459	420	428	449
No. of litters with viable fetuses	25	26	24	25
No. of live fetuses per litter	8.9	8.3	8.3	8.7
Fetal body weights (g)				
- males	34.8	34.9	34.9	34.8
- females	34.9	33.4	34.7	33.9
External examination (no. of fetuses/litters)	223/25	215/26	199/24	217/25
- total malformed (no. of fetuses/litters)	0/0	0/0	1/1	0/0
Craniofacial examination (no. of fetuses/litters)	117/25	114/26	105/24	114/25
- total malformed (no. of fetuses/litters)	0/0	0/0	0/0	0/0
Visceral examination (no. of fetuses/litters)	223/25	215/26	199/24	217/25
- total malformed (no. of fetuses/litters)	1/1	3/2	3/2	2/2
Skeletal examination (no. of fetuses/litters)	223/25	215/26	199/24	217/25
- total malformed (no. of fetuses/litters)	0/0	0/0	0/0	0/0
Dose in dams (mg/kg bw per day), GD 27	0	1.2	5.6	31.5
Plasma $AUC_{24\,h}$ in dams (µg·h/ml), GD 27	0	20.5	107	599
Plasma concentration (µg/g), GD 28				
- dams	0	0.7	3.7	23.9
- fetuses	0	0.6	3.5	21.2

From Rasoulpour & Brooks (2009b)
AUC, area under the curve; GD, gestation day; * $P < 0.05$

With the exception of one F_0 female in the control and 750 ppm groups euthanized on LD 3 due to total litter loss, all females survived to the scheduled necropsies. No test substance–related maternal macroscopic findings were noted.

Lower mean body weight gains (24.2%) and feed consumption (7.3%) were noted in the 750 ppm group during the gestation exposure period compared with the control group (Table 33). Corresponding incidences of decreased defecation were noted for three females in this group. Although mean body weights remained within 2.9% of control group values throughout gestation, the reductions in mean body weight gains and feed consumption were attributed to test substance exposure. Mean body weights, body weight gains and feed consumption in the 750 ppm group were similar to those of the control group during LDs 1–4.

No test substance–related effects were observed on the mean number of offspring born, offspring survival or the general physical condition of the offspring.

Table 33. Summary of selected findings in a special developmental toxicity study in rabbits

	0 ppm	750 ppm
Body weight change (g)		
- GDs 7–10	−1	−12
- GDs 10–13	81	64
- GDs 13–16	153	121
- GDs 7–28	413	313
- LDs 1–4	33	67
Feed consumption (g/day)		
- GDs 7–10	150	137
- GDs 10–13	150	132
- GDs 13–16	150	136
Gestation length (days)	31.5	31.3
Litter size, PND 0	10.9	10.6
Live litter size, PND 0	10.8	9.8
Postnatal survival (% per litter)		
- PND 0, relative to number born	99.3	93.8
- PND 0 to PND 1	97.8	99.2
- PND 1 to PND 4	74.2	78.4
- Birth to PND 4	72.7	71.6

From Kuhl (2009)
GD, gestation day; LD, lactation day; PND, postnatal day

The NOAEL for maternal toxicity was lower than 750 ppm (equal to 29 mg/kg bw per day), the only dose tested, based on decreased body weight gain and feed consumption.

The NOAEL for neonatal survival was 750 ppm (equal to 29 mg/kg bw per day), the only dose tested (Kuhl, 2009).

2.6 Special studies

(a) Neurotoxicity

In an acute neurotoxicity study conducted in accordance with OECD test guideline 424, groups of 10 male and 10 female F344/DuCrl rats received sulfoxaflor (purity 95.6%) by oral gavage at a dose level of 0, 7.5, 75 or 750 mg/kg bw. The test substance was administered in a 0.5% aqueous Methocel cellulose vehicle at a volume of 10 ml/kg bw. The dose levels were selected on the basis of the results from a pilot study conducted at dose levels of 500 and 750 mg/kg bw using five female rats per dose. Body weights were recorded and a FOB and test for motor activity were conducted pre-exposure (baseline) and on the day of dosing (day 1, time of peak effect), day 8 and day 15. The FOB included hand-held and open-field observations as well as measurements of grip performance, landing foot splay and rectal temperature. Clinical observations were conducted on days 2, 3 and 4. At the end of the study, all rats from the control and high-dose groups and five rats of each sex from the low- and mid-dose groups were perfused for histopathological evaluation of the central and peripheral nervous systems. A second motor activity study at dose levels of 0, 2.5, 7.5 and 25 mg/kg bw was conducted to investigate whether an apparent decrease in motor activity at 7.5 mg/kg bw was reproducible or treatment related and to establish a clear NOAEL. Motor activity was the only end-point examined in this study phase.

In the pilot study at 750 mg/kg bw, two rats had convulsions 1 hour after dosing, and four had decreased activity and muscle tremors beginning 2–4 hours after dosing. Other clinical signs observed in these rats included hindlimb splay, increased reactivity, walking on toes and perineal urine soiling. The highest frequency of these observations occurred 2–4 hours after dosing. One rat given 750 mg/kg bw had no treatment-related clinical signs. At 500 mg/kg bw, treatment-related clinical signs included decreased activity, muscle tremors, hindlimb splay, lacrimation and perineal urine soiling. Similarly, the highest frequency of these observations occurred 2–4 hours after dosing. Based on these results, the time of peak effect was determined to be between 2 and 4 hours after dosing, and the time selected to begin behavioural testing in the full study was 2.5 hours after dosing.

In the main study, one female rat given 750 mg/kg bw died following dosing on day 1, but the cause of death could not be determined. Treatment-related clinical findings were noted in males and females at 750 mg/kg bw on day 2, 3 or 4 and included decreased or absent faeces, red perioral soiling and perineal urine soiling (females only). There was a statistically significant treatment-related decrease in body weight at 750 mg/kg bw when compared with controls on days 8 and 15, which was more prominent in males than in females.

Treatment-related categorical observations on day 1 in males and females at 750 mg/kg bw included increased incidences of muscle tremors and twitches, convulsions, splayed hindlimbs and perineal urine soiling.

Treatment-related ranked FOB observations on day 1 in males and females at 750 mg/kg bw were as follows: increased lacrimation and salivation, decreased pupil size and response to touch, increased level of urination (females only) and decreased level of open-field activity and gait abnormalities. There were no changes in either treatment-related categorical or ranked FOB observations in males or females at 750 mg/kg bw on day 8 or day 15 or in males or females at 7.5 or 75 mg/kg bw during any FOB time point.

There was a treatment-related decrease in rectal temperature at 750 mg/kg bw when compared with controls on day 1, which was not present in the subsequent examinations on days 8 and 15. There were no treatment-related effects in grip performance or landing foot splay.

There was a treatment-related decrease in the day 1 total motor activity and an effect on the distribution of motor activity counts of males and females at 75 or 750 mg/kg bw. The effect on total motor activity of animals at 7.5 mg/kg bw was considered equivocal on day 1. There were no effects on motor activity on day 8 or 15 in rats of any dose group. In the follow-up motor activity study, there were no treatment-related effects on total motor activity or on the distribution of motor activity counts for males and females at 2.5, 7.5 or 25 mg/kg bw when compared with controls (Table 34).

There were no treatment-related gross or histopathological findings in the central or peripheral nervous system.

The NOAEL for acute neurotoxicity was 25 mg/kg bw, based on decreased motor activity at 75 mg/kg bw. The NOAEL for neuropathology was 750 mg/kg bw, the highest dose tested (Marty, Andrus & Stebbins, 2010).

In a developmental neurotoxicity study conducted in accordance with OECD test guideline 426, groups of 25 bred female Crl:CD(SD) rats were given diets containing sulfoxaflor (purity 95.6%) at a concentration of 0, 25, 100 or 400 ppm from GD 6 through LD 21. The mean dose at 25, 100 and 400 ppm was equal to 1.8, 7.1 and 27.7 mg/kg bw per day through gestation and 1.9, 7.6 and 29.8 mg/kg bw per day through lactation, respectively.

All animals were observed twice daily for appearance and behaviour. Clinical observations, body weights and feed consumption were recorded at appropriate intervals during gestation and lactation. In addition, detailed clinical observations were conducted out of the home cage on all dams in each group on GDs 10 and 15 and on LDs 10 and 21. All females were allowed to deliver and rear

Table 34. Summary of selected findings in an acute neurotoxicity study in rats

	Males				Females			
Main study								
Dose (mg/kg bw per day)	0	7.5	75	750	0	7.5	75	750
Body weight (g)								
- day 2	131.3	133.2	132.6	119.7*	89.1	89.6	86.4	82.7
- day 8	141.5	144.8	142.9	127.7*	96.9	97.8	94.5	91.6
- day 15	161.2	164.0	161.7	145.7*	111.1	112.8	108.2	106.4
Rectal temperature (°C), day 1	37.5	37.2	37.2	34.5*	37.9	38.1	38.1	34.7*
Motor activity (activity counts), pre-exposure	17.6	16.1	18.8	17.7	19.3	19.8	17.2	19.1
- day 1	18.1	12.9*	12.4*	3.0*	19.9	17.9*	10.8*	7.3*
- day 8	15.8	16.8	19.6	15.4	20.8	22.8	20.4	19.8
- day 15	16.2	15.3	16.7	15.0	17.9	24.9	23.7	18.4
Follow-up motor activity study								
Dose (mg/kg bw per day)	0	2.5	7.5	25	0	2.5	7.5	25
Motor activity (activity counts), pre-exposure	16.7	13.2	14.0	13.1	14.8	14.6	15.6	16.1
- day 1	12.2	13.0	14.8	13.0	17.3	17.7	17.3	17.9
- day 8	16.9	19.3	19.0	19.6	25,7	23.1	25.0	24.7
- day 15	21.0	18.6	19.6	22.3	25.8	25.6	28.6	26.7

From Marty, Andrus & Stebbins (2010)
* $P < 0.02$ (treatment by time interaction, with male and female data considered together)

their offspring to LD 21. F_0 females that failed to deliver or with total litter loss were necropsied on post-mating day 25 or within 24 hours of total litter loss, respectively. The liver and kidneys from all F_0 females were weighed at necropsy. Clinical observations, body weights and sexes were recorded for the F_1 pups at appropriate intervals. Pre-weaning developmental landmarks (surface righting response and eye opening) were evaluated for all available F_1 pups. On PND 4, litters were culled to eight pups per litter. If a litter consisted of fewer than six pups or failed to meet sex ratio criteria (at least three pups of each sex), the litter was not used for neurobehavioural or neuropathological evaluation, and the dam and litter were necropsied on PND 4. Following culling, a subset of 20 pups of each sex per group (subset A) was assigned to detailed clinical observations (PNDs 4, 11, 21, 35, 45 and 60), auditory startle response (PNDs 20 and 60), locomotor activity (PNDs 13, 17, 21 and 61), learning and memory (PND 62) and brain weight evaluations (PND 72). From this subset, 10 pups of each sex from the control and 400 ppm groups were selected for neuropathological and brain morphometric evaluations on PND 72. A second subset of 20 pups of each sex per group (subset B) was selected for assessment of learning and memory (PND 22). A third subset of 20 pups of each sex per group (subset C) was selected for brain weight evaluations on PND 21; of these, 10 pups of each sex from the control and 400 ppm groups were selected for neuropathological and brain morphometric evaluations on PND 21. Indicators of sexual development (balanopreputial separation and vaginal patency) were evaluated for all F_1 animals in subset A. All F_1 animals not selected for behavioural evaluations were euthanized and necropsied on PND 21. F_1 animals selected for learning and memory assessment on PND 22 were necropsied following completion of these assessments.

There were no treatment-related mortalities in the F_0 maternal animals during the study, and no test substance–related clinical findings were noted during the daily examinations. Total litter loss was noted for two dams in the control group on LDs 0 and 9 and for one dam at 400 ppm on LD 2. Detailed clinical observations as well as maternal body weights and feed consumption during gestation and lactation were unaffected by treatment.

There were no treatment-related differences noted between groups when comparing the mean length of gestation, the process of parturition and internal macroscopic pathological findings. The mean numbers of former implantation sites and sites that were unaccounted for as well as maternal kidney and liver weights were similar across groups.

There were no treatment-related effects on the mean number of pups born, live litter size or the percentage of males at birth at any maternal exposure level. However, F_1 pup toxicity was expressed at 400 ppm by a statistically significant reduction in postnatal survival from birth to PND 4 compared with the control group (Table 35). In addition, malrotation of the left forelimb was noted for two pups in the same litter at 400 ppm during the week prior to weaning (on PNDs 14, 17 and/or 21); this observation was not apparent on PND 1, 4, 7 or 11 for either of these pups, both of which survived to the scheduled euthanasia on PND 21.

Mean pup body weights at 400 ppm were 11.8% and 6.5% lower than those of the control group at birth (PND 1) and on PND 4, respectively. The reduced pup body weights resulted in a statistically significant delay in surface righting response for pups at 400 ppm (Table 35). Pup body weights in the 400 ppm group did not differ from the control group values on PND 7 or later time points. The decrease in postnatal survival at 400 ppm is consistent with results from a previous pilot reproduction study, in which dietary exposures of 500 and 1000 ppm resulted in decreased pup survival. Postnatal survival and pup body weights and body weight gains in the 25 and 100 ppm groups were unaffected by maternal test substance exposure. The ages of attainment of surface righting response at 25 and 100 ppm and eye opening at 25, 100 and 400 ppm were similar to those of the control group. The attainment of sexual developmental landmarks (balanopreputial separation and vaginal patency) was unaffected by maternal test substance exposure.

No remarkable clinical observations or macroscopic findings were noted for F_1 animals at any exposure level. No treatment-related effects were observed for F_1 animals with respect to detailed clinical observations, locomotor activity, auditory startle response, and learning and memory. Furthermore, there were no treatment-related effects on brain weights, measurements and morphometric parameters or histopathology of the brain and/or central and peripheral nervous systems for F_1 animals on PNDs 21 and 72.

The NOAEL for maternal toxicity and reproductive toxicity (process of parturition and duration of gestation) was 400 ppm (equal to 28.8 mg/kg bw per day), the highest dose tested.

The NOAEL for developmental neurotoxicity was 400 ppm (equal to 28.8 mg/kg bw per day), as there were no signs of developmental neurotoxicity at the highest dose tested.

The NOAEL for neonatal toxicity was 100 ppm (equal to 7.4 mg/kg bw per day), based on the reduction in postnatal survival and pup body weights at 400 ppm (equal to 28.8 mg/kg bw per day) (Beck, 2010).

(b) Mechanistic studies on liver tumour induction in mice and rats

Mice

A mechanistic study was conducted to obtain information on the potential MOA responsible for the liver weight increases observed in mice and rats after repeated exposure to sulfoxaflor. For this purpose, specific gene expression was assessed by real-time polymerase chain reaction (RT-PCR) in liver samples from female CD-1 mice exposed to 0 or 4500 ppm sulfoxaflor in the diet for 4 days in a previously conducted palatability pilot study (Thomas & Dryzga, 2010). In total, eight genes were selected for this study. Five genes, primarily *Cyp2b10*, but with four additional genes, were chosen to address whether sulfoxaflor induces a phenobarbital-like gene expression response. Two genes that are also induced by phenobarbital were selected specifically to investigate the effect on blood cholesterol seen in sulfoxaflor-treated rodents. One gene, *Cyp4a10*, was included as a marker of peroxisome proliferator-activated receptor alpha (PPARα) to examine this potential MOA.

Table 35. Summary of selected findings in a developmental neurotoxicity study in rats

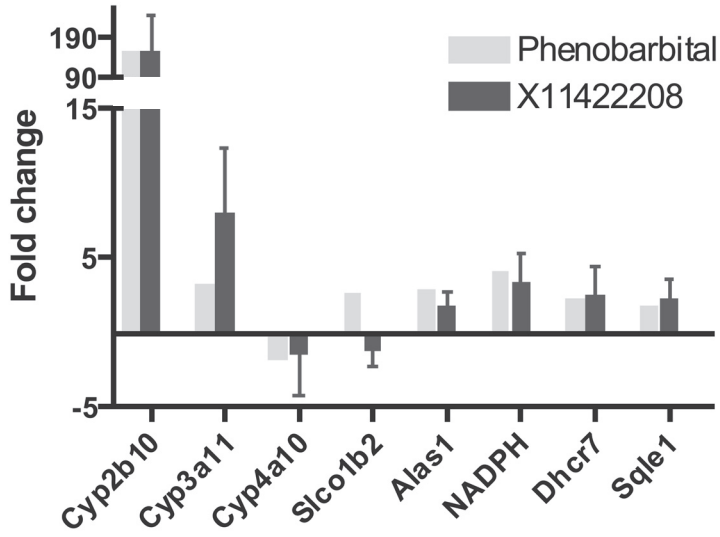
	0 ppm	25 ppm	100 ppm	400 ppm
No. of females delivered	25	25	24	25
Mean litter size, at birth	14.8	15.2	15.3	15.2
Live litter size, PND 0	14.2	15.0	15.1	14.9
Postnatal survival (%)				
- PNDs 0–1	99.5	98.9	96.0	86.9*
- PNDs 1–4	99.8	99.8	98.4	87.2
- birth to PND 4	93.0	97.9	93.2	76.5**
No. of pups/litters found dead or euthanized	24/8	6/5	17/11	59/15
No. of pups/litters with malrotation of left forelimb	0/0	0/0	0/0	2/1
Pup body weight (g)				
- PND 1	6.8	6.7	6.7	6.0**
- PND 4	9.2	9.1	9.2	8.6**
- PND 7	14.3	14.5	14.7	14.0
- PND 21	43.9	45.7	45.2	43.8
Surface righting response (PND)	5.3	5.2	5.2	6.3**
Eye opening (PND)	14.8	15.1	14.8	14.9
Balanopreputial separation (PND) / body weight (g)	46.9/247	46.3/247	46.1/245	47.6/236
Vaginal patency (PND) / body weight (g)	32.7/104	32.9/106	32.7/105	32.6/105
Locomotor activity (counts), both sexes combined				
- PND 13	1585	1499	1688	2411
- PND 17	3130	2687#	2855#	2339
- PND 21	2498	2198	2937	2790
- cumulative, PNDs 13–21, both sexes combined	7213	6385	7480	7540
- PND 61, both sexes combined	5559	4691	5613	4723
- PND 61, males	5043	4306	4527	4499
- PND 61, females	6050	5076#	6698	4947

From Beck (2010)
PND, postnatal day; * $P < 0.05$; ** $P < 0.01$; # $P < 0.01$ (treatment by time interaction)

In addition, cell proliferation (seen as an early response to phenobarbital treatment) was assessed by Ki-67 immunohistochemical staining in liver tissue from female CD-1 mice from the mouse palatability study (Thomas & Dryzga, 2010; 0, 3000 and 4500 ppm group) and male and female F344/DuCrl rats from a 28-day rat study (Yano et al., 2009b; 0 and 2000 ppm group).

Sulfoxaflor-treated mice showed gene expression changes similar to those reported in the literature following phenobarbital exposure. The primary phenobarbital-like marker gene, *Cyp2b10*, was induced 148.5-fold. In addition, three of the four remaining phenobarbital-like response genes (*Cyp3a11*, *Alas1* and *NADPH-Cyp-reductase*) were induced 7.85-, 1.15- and 3.18-fold, respectively. The remaining phenobarbital-like gene, *Slco1b2*, was not induced in sulfoxaflor-treated mice. Sulfoxaflor stimulated the cholesterol synthesis–related genes, *Dhcr7* and *Sqle1* (2.42- and 2.05-fold, respectively), at levels similar to those reported in the literature following phenobarbital exposure. The lack of induction for both *Cyp4a10* and an internal homeostasis control gene, *Scd1*, indicates that sulfoxaflor is not acting as a peroxisome proliferator (Figure 2).

Figure 2. Sulfoxaflor (X11422208) and phenobarbital gene expression response in mice (phenobarbital gene expression values are from the literature)



From Geter & Kan (2008)

Cell proliferation in mice was significantly increased in both the centrilobular and midzonal regions at 3000 ppm (19- and 5-fold, respectively), whereas no significant changes in proliferation were seen in the periportal region at 3000 ppm or in any region at 4500 ppm. The lack of significant proliferation response at 4500 ppm was most probably due to the shortened length of exposure (3 days) when compared with the 3000 ppm group (7 days). The proliferation response was accompanied by greater numbers of hepatocyte mitotic figures in the 3000 ppm group than in the 4500 ppm group.

In rats following 28-day treatment at 2000 ppm, a significant increase in proliferation was seen only in the centrilobular region (1.7- or 3-fold in males or females, respectively).

These findings suggest that the MOA responsible for liver weight increases observed in sulfoxaflor-treated mice and rats is phenobarbital-like (Geter & Kan, 2008).

In a subsequent mechanistic study conducted to determine if a phenobarbital-like MOA was responsible for the liver weight increases seen in mice after repeated exposure to sulfoxaflor, groups of five male and five female Crl:CD-1 (ICR) mice per group were given diets containing sulfoxaflor (purity 95.6%) at a concentration of 0, 500 or 750 ppm (males) or 0, 1000 or 1500 ppm (females) for 7 days. The dietary concentrations were equal to 0, 89 and 128 mg/kg bw per day for males and 0, 211 and 323 mg/kg bw per day for females. End-points evaluated were daily cage-side observations, body and liver weights, feed consumption, serum clinical chemistries, targeted gene expression (quantitative RT-PCR using TaqMan gene expression assays), liver cytochrome P450 enzyme activity and hepatocellular proliferation using 5-bromo-2′-deoxyuridine (BrdU) and Ki-67 immunohistochemical techniques. Four nuclear receptors that are primarily responsible for xenobiotic-induced liver weight increase were investigated: the aryl hydrocarbon receptor (AhR; *Cyp1a*), constitutive androstane receptor (CAR; *Cyp2b*), pregnane X receptor (PXR; *Cyp3a*) and PPARα (*Cyp4a*).

In addition, archived liver samples from previously conducted 28-day and 90-day toxicity studies in mice (Thomas et al., 2008, 2010a) were analysed for targeted gene expression, liver enzyme activity and hepatocellular proliferation (Ki-67).

All mice in the 7-day study survived the treatment period. There were no statistically identified differences in body weights of males or females at any dose level. Feed consumption was not affected by treatment in males, but was reduced at 1000 and 1500 ppm in females (12% and 10%, respectively).

Males exposed to 750 ppm had a statistically significant increase (17%) in relative liver weights compared with controls. Females exposed to 1000 and 1500 ppm had a statistically significant increase in relative (38% and 43%, respectively) and absolute (43% and 47%, respectively) liver weights compared with controls (Table 36). These liver weight increases were accompanied by centrilobular and midzonal hepatocyte hypertrophy, with very slightly increased cytoplasmic eosinophilia (altered tinctorial properties consistent with enzyme induction and/or smooth endoplasmic reticulum increase). There were very slight increases in the number of mitotic hepatocytes in some males at 500 or 750 ppm and in the majority of females at 1000 or 1500 ppm. In addition, minimal focal or very slight multifocal individual cell necrosis of hepatocytes occurred in some males at 500 or 750 ppm and in females at 1000 or 1500 ppm, characterized by the presence of one or two necrotic hepatocytes (minimal focal) or five or six scattered necrotic hepatocytes (very slight multifocal) in the entire liver section. Analysis by Oil Red O stain demonstrated slightly increased amounts of lipid in the hepatocytes in males at 750 ppm sulfoxaflor; however, no changes were observed in females.

In the 7-day study, *Cyp2b10* expression, considered to be the prototypical gene response following phenobarbital exposure through activation of CAR, was induced 42.1- and 54.8-fold in males at 500 and 750 ppm, respectively, and 20.0- and 30.8-fold in females at 1000 and 1500 ppm, respectively, relative to controls. In the 28-day and 90-day studies, *Cyp2b10* gene expression in males was increased 61.7-fold (300 ppm) and 56.5-fold (750 ppm), respectively, and in females at 1500 ppm, 93.9- and 53.9-fold, respectively (Table 36).

Cyp3a11, a phenobarbital- and PXR-related gene, was significantly elevated in males at 750 ppm after 7 and 90 days (2.7- and 2.8-fold, respectively), whereas in female mice, it was significantly elevated at all doses and time periods (range of 3.4- to 6.6-fold). 7-Pentoxyresorufin-*O*-deethylase (PROD) and benzyloxyresorufin-*O*-debenzylase (BROD) liver enzyme activities, which give a measure of *Cyp2b* enzyme induction, were elevated in both male and female mice at all time points (range of 2.6- to 9.5-fold).

AhR-related 7-ethoxyresorufin-*O*-deethylase (EROD) liver enzyme activity was slightly elevated at all time points in both male and female mice; however, the degree of induction was mild (none greater than 2.3-fold) and was most likely associated with the large induction of *Cyp2b* enzyme. *Cyp4a10*, a PPARα-related gene, was not significantly altered in this study.

BrdU analysis in the 7-day study showed that males at 500 ppm had elevated proliferation in the centrilobular region and at 750 ppm in both the centrilobular and midzonal regions, whereas females at 1000 and 1500 ppm showed significant induction in all three regions. Using Ki-67 analysis, males at 500 and 750 ppm showed significant induction in the centrilobular region; however, unlike the BrdU analysis, increased proliferation was not observed at 750 ppm in the midzonal region. Ki-67 analysis in females showed no evidence for increased proliferation at any dose or zone.

In the 28- and 90-day studies, Ki-67 analysis of hepatocellular proliferation showed no induction at either time point in male or female mice.

Based upon these results, the MOA responsible for the increased liver weight in mice administered sulfoxaflor was phenobarbital-like, as evidenced by the CAR- and PXR-related molecular, enzymatic and proliferative responses. These data support the conclusion that sulfoxaflor may be an agonist ligand for CAR, and this activation results in the observed phenobarbital-like response and increased liver weight (Geter et al., 2010a).

A mechanistic study was conducted to characterize the induction profile of sulfoxaflor in the livers of C57BL/6J mice in order to provide comparative data to previously conducted studies

Table 36. Summary of selected findings in a mechanistic study in mice

	Males			Females		
7-day study						
Dietary concentration (ppm)	0	500	750	0	1000	1500
Dose (mg/kg bw per day)	0	89	128	0	211	323
Body weight (g)	32.4	31.5	31.5	23.3	24.0	23.8
Liver weight, absolute (g)	1.79	1.80	2.03	1.05	1.50*	1.54*
Liver weight, relative (% of body weight)	5.51	5.72	6.45*	4.50	6.24*	6.45*
Hepatocellular hypertrophy	0/5	0/5	5/5	0/5	4/5	5/5
Mitotic alteration of hepatocytes	0/5	2/5	3/5	2/5	4/5	5/5
Necrosis, single cell, focal/multifocal	0/5	2/5	3/5	0/5	1/5	1/5
Targeted gene expression (fold change)						
- *Cyp 2b10*	1	42.1*	54.8*	1	20.0*	30.8*
- *Cyp 3a11*	1	1.6	2.7*	1	4.0*	6.6*
- *Cyp 4a10*	1	1.2	1.0	1	−5.6	−3.3
Liver enzyme activity						
- EROD (pmol/min per milligram protein)	20.3	30.1*	33.3*	10.5	23.3*	23.0*
- PROD (pmol/min per milligram protein)	1.2	5.1*	5.4*	2.8	13.9*	13.8*
- BROD (pmol/min per milligram protein)	2.7	18.0*	19.7*	2.2	7.7*	8.6*
Liver cell proliferation (BrdU)						
- centrilobular (proliferation index)	2.3	6.2*	7.3*	8.9	36.7*	43.3*
- midzonal (proliferation index)	1.5	4.9	5.6*	8.4	30.0*	39.1*
- periportal (proliferation index)	2.6	3.7	3.8	8.2	22.1*	31.0*
Liver cell proliferation (Ki-67)						
- centrilobular (proliferation index)	0.5	1.1*	1.1*	0.8	2.4	1.8
- midzonal (proliferation index)	0.5	1.0	0.8	0.9	2.0	2.1
- periportal (proliferation index)	0.7	0.5	0.6	0.4	0.8	0.6
28-day study						
Dietary concentration (ppm)	0	300	—	0	—	1500
Dose (mg/kg bw per day)	0	43.9	—	0	—	273
Targeted gene expression (fold change)						
- *Cyp 2b10*	1	61.7*	—	1	—	93.9*
- *Cyp 3a11*	1	1.5	—	1	—	5.6*
- *Cyp 4a10*	1	−1.6	—	1	—	−1.8
Liver enzyme activity						
- EROD (pmol/min per milligram protein)	16.8	23.8*	—	12.7	—	22.9*
- PROD (pmol/min per milligram protein)	1.8	8.8*	—	2.7	—	6.9*
- BROD (pmol/min per milligram protein)	2.3	21.4*	—	3.3	—	14.9*
90-day study						
Dietary concentration (ppm)	0	—	750		—	1500
Dose (mg/kg bw per day)		—	98		—	247
Targeted gene expression (fold change)						
- *Cyp 2b10*	1	—	56.5*	1	—	53.9*
- *Cyp 3a11*	1	—	2.8*	1	—	3.4*
- *Cyp 4a10*	1	—	−3.6	1	—	−2.3
Liver enzyme activity						
- EROD (pmol/min per milligram protein)	20.1	—	24.8*	16.4	—	24.5*
- PROD (pmol/min per milligram protein)	4.0	—	15.5*	5.1	—	20.0*
- BROD (pmol/min per milligram protein)	5.2	—	22.0*	6.1	—	24.4*

From Geter et al. (2010a)

BrdU, 5-bromo-2′-deoxyuridine; BROD, benzyloxyresorufin-*O*-debenzylase; EROD, 7-ethoxyresorufin-*O*-deethylase; PROD, 7-pentoxyresorufin-*O*-deethylase; * $P < 0.05$

in CD-1 mice. If comparable, these preliminary data would be used for an extensive study using C57BL/6J CAR/PXR knockout and "humanized" mice. Groups of five male C57BL/6J mice were given diets containing sulfoxaflor (purity 95.6%) at a concentration of 0, 750 or 1500 ppm (equal to 0, 160 and 310 mg/kg bw per day) for 7 days. End-points evaluated were daily clinical observations, body weights, body weight gain, feed consumption, serum clinical chemistry, targeted gene expression (quantitative RT-PCR using TaqMan gene expression assays; and sodium dodecyl sulfate–polyacrylamide gel electrophoresis [SDS-PAGE] and western blotting), protein quantification of liver microsomes, liver cytochrome P450 enzyme activity and hepatocellular proliferation.

There were no treatment-related clinical observations or effects on body weight or body weight gain throughout the study. There was a treatment-related increase in absolute and relative liver weights following 7 days of exposure to sulfoxaflor. Absolute liver weights at 750 and 1500 ppm groups were 117% and 128% of those of controls, respectively, and relative liver weights were 117% and 140% of those of controls, respectively. Despite this increase in liver weight, there was a lack of overt hepatotoxicity at either dose level of sulfoxaflor, as evidenced by the absence of toxicologically significant increases in plasma AST, cholesterol and triglycerides. ALT was increased in a dose-dependent manner (2-fold at the high dose level); however, this finding was not deemed to be of toxicological significance, as values were all within the historical control range.

Sulfoxaflor at 750 ppm and 1500 ppm elicited a 3- and 5-fold increase in total hepatic P450 content, respectively, a 33-fold increase in PROD activity (at both concentrations), a 47- and 82-fold increase in BROD activity, respectively, and a 4- and 7-fold increase in benzyloxyquinoline debenzylase (BQ) activity, respectively.

Gene expression results indicated the presence of *Cyp2b10* messenger ribonucleic acid (mRNA) in both treatment groups, but not controls. As *Cyp2b10* is not constitutively expressed, a fold change over control values cannot be calculated for treated groups, although it can be seen that there was a large difference from controls, and the change from treatment at 750 ppm to 1500 ppm was 9.2-fold. Unlike *Cyp2b10*, *Cyp3a11* is constitutively expressed in the mouse, and results can be expressed as a relative fold change over control values. Sulfoxaflor at 750 and 1500 ppm elicited a 2.4- and 5.6-fold increase in *Cyp3a11* relative to controls. These gene expression data were confirmed by SDS-PAGE and western blotting, which demonstrated that sulfoxaflor clearly induced *Cyp2b10* and *Cyp3a11*.

In conclusion, it would appear that sulfoxaflor exerts its enzyme induction properties via CAR and possibly also PXR (Elcombe, 2010).

A mechanistic study was conducted to investigate the MOA for sulfoxaflor-induced liver effects by use of dual CAR-PXR knockout and "humanized" mouse models. The aim of the study was to investigate 1) if CAR or PXR mediates sulfoxaflor-induced hypertrophy and hyperplasia in mice and 2) if the human receptors (CAR and PXR) support these processes to a similar extent as the murine receptors. The mouse models used were wild-type C57BL/6J (WT) mice, C57BL/6J mice null for PXR and CAR (PXRKO/CARKO) and C57BL/6J mice humanized for PXR and CAR (hPXR/hCAR).

Groups of 10 male mice of each strain were given diets containing sulfoxaflor (purity 95.6%) at a concentration of 0 or 750 ppm for 7 days. The mean doses at 750 ppm were equal to 116, 120 and 99 mg/kg bw per day for WT, PXRKO/CARKO and hPXR/hCAR mice, respectively. End-points evaluated were daily clinical observations, body weights, body weight gain, feed consumption, plasma clinical chemistry, targeted gene expression (quantitative RT-PCR using TaqMan gene expression assays; and SDS-PAGE and immunoblotting), protein quantification of liver microsomes, liver cytochrome P450 enzyme activity (PROD, BROD, BQ), hepatocellular proliferation using nuclear incorporation of BrdU and liver histopathology.

There were no treatment-related clinical observations or effects on body weight or body weight gain in any strain of mice. There were treatment-related increases in absolute and relative

liver weights in WT and hPXR/hCAR mice (absolute liver weights: 124% and 109% of controls, respectively; relative liver weights: 125% and 112% of controls, respectively), but not in the PXRKO/CARKO animals.

In WT mice, sulfoxaflor treatment increased hepatocellular proliferation (by approximately 4-fold), but no such changes in proliferation were seen in either the hPXR/hCAR or PXRKO/CARKO mice. Treatment-related hepatocyte hypertrophy was observed in WT and hPXR/hCAR mice, whereas increased mitotic figures were observed only in WT mice. Neither hepatocyte hypertrophy nor increased mitotic figures were seen in PXRKO/CARKO mice.

In agreement with results of the pilot study (Elcombe, 2010), sulfoxaflor behaved as a phenobarbital-like inducer in WT mice. This was demonstrated by marked induction of total cytochrome P450 content and markedly increased PROD and BROD activities (approximately 33- and 36-fold, respectively). Under the same treatment, induction of PROD and BROD in hPXR/hCAR mice was marginal (approximate increases of 2- and 3-fold, respectively), whereas neither PROD nor BROD activity was induced in PXRKO/CARKO mice.

These results were confirmed by the increased expression of *Cyp2b10* mRNA demonstrated by RT-PCR and by immunoblotting data showing increases in Cyp2b10 protein. As hepatic *Cyp2b10* is not constitutively expressed in WT C57BL/6J mice, a fold change in response to sulfoxaflor treatment cannot be calculated over control values, although it can be seen that there is a large difference from WT controls (mean threshold cycle [Ct] 29.8 ± 0.5 versus mean Ct > 35, respectively; Ct values > 35 are indicative of mRNA levels below the limits of detection for the assay) (Table 37). In contrast, basal *Cyp2b10* mRNA was expressed in the hPXR/hCAR animals, with a marginal (approximately 3.9-fold) increase in *Cyp2b10* mRNA observed following sulfoxaflor treatment. *Cyp2b10* mRNA levels were undetectable in control PXRKO/CARKO animals and remained so upon exposure to sulfoxaflor. These data indicate that *Cyp2b10* transcription is markedly upregulated by sulfoxaflor in WT mice, but not in hPXR/hCAR or PXRKO/CARKO mice.

Sulfoxaflor-mediated *Cyp3a11* induction, as determined by BQ activity (Cyp3a selective reaction), RT-PCR and immunoblotting, was observed in the humanized and WT mice to similar extents, but was not seen in the PXRKO/CARKO mice (Table 37).

In conclusion, sulfoxaflor exhibited markedly more activity towards the mouse CAR than the human CAR and relatively weak activity towards the mouse and human PXR. Hence, the difference in hepatic response between wild-type and humanized mice in this study is considered to be mediated via CAR. Furthermore, the data show that the human CAR and PXR support sulfoxaflor-induced hypertrophy but not hyperplasia. The study demonstrates that sulfoxaflor, like phenobarbital, acts via a CAR-mediated MOA (Ross, 2010).

Rats

In a mechanistic study conducted to determine if a phenobarbital-like MOA was responsible for the liver weight increases seen in rats after repeated exposure to sulfoxaflor and to obtain information on the time– and dose–response relationships for the effect, groups of five male and five female F344/DuCrl rats per group were given diets containing sulfoxaflor (purity 96.6%) at a concentration of 0, 100, 750 or 1500 ppm for 3 or 7 days. These dietary concentrations were equal to 0, 8.85, 60.3 and 99.2 mg/kg bw per day for males and 0, 7.83, 50.6 and 83.3 mg/kg bw per day for females necropsied on day 3, respectively, and equal to 0, 8.02, 58.6 and 102 mg/kg bw per day for males and 0, 7.74, 53.1 and 94.4 mg/kg bw per day for females necropsied on day 7, respectively. The primary end-points examined in this study were liver weight, targeted gene expression (quantitative RT-PCR using TaqMan gene expression assays), liver cytochrome P450 enzyme activity (EROD, PROD, BROD) and hepatocellular proliferation (Ki-67 immunohistochemical staining). Four nuclear receptors that are primarily responsible for xenobiotic-induced liver weight increase were investigated: AhR, CAR, PXR and PPARα.

Table 37. Summary of selected findings in a mechanistic study in mice

	WT		PXRKO/CARKO		hPXR/hCAR	
	Dietary concentration (ppm)					
	0	750	0	750	0	750
Body weight (g)	22.4	22.2	24.1	24.7	25.5	24.8
Liver weight, absolute (g)	0.99	1.22**	1.13	1.21	1.23	1.35
Liver weight, relative (% of body weight)	4.40	5.50**	4.69	4.89	4.84	5.42**
Hepatocellular hypertrophy	0/10	10/10	0/10	0/10	0/10	10/10
Mitotic alteration of hepatocytes	1/10	7/10	0/10	0/10	0/10	1/10
BrdU-positive hepatocytes (%)	2.41	8.79**	0.38	0.53	0.58	0.88
PROD (pmol/min per milligram protein)	3.3	110**	4.7	4.8	11.2	27.9**
BROD (pmol/min per milligram protein)	7.6	274**	20.3	33.3	83.0	241**
BQ (nmol/min per milligram protein)	1.8	4.8**	3.2	3.3	3.9	8.1**
Total P450 (nmol/mg protein)	0.45	0.86**	0.51	0.51	0.64	0.90**
Cyp2b10; average Ct value	> 35	29.8	>3 5	> 35	33.6	31.7
- fold change, relative to control	—	ND	—	ND	—	3.9
Cyp3a11; average Ct value	21.1	20.1	21.3	22.5	20.2	18.5
- fold change, relative to control	—	2.1	—	−2.5	—	3.4

From Ross (2010)
BQ, benzyloxyquinoline debenzylase; BrdU, 5-bromo-2′-deoxyuridine; BROD, benzyloxyresorufin-*O*-debenzylase; Ct, threshold cycle; ND, not determined; PROD, 7-pentoxyresorufin-*O*-deethylase; ** $P < 0.01$

All rats survived the treatment period. Body weight gains and feed consumption were reduced at 750 and 1500 ppm. Relative liver weights were increased for males and females in the 1500 ppm group at 3 days (14% and 3%, respectively) and at 750 and 1500 ppm at 7 days (11% and 23% for males and 6% and 18% for females, respectively) (Table 38).

Cyp*2b1* gene expression, the prototypical gene response following phenobarbital exposure, was induced over 800-fold in both male and female rats exposed to 1500 ppm sulfoxaflor for 3 and 7 days. To further examine similarities in phenobarbital-like response, *Cyp2b2* and *Cyp3a3* (CAR- and PXR-related genes, respectively) expression levels, together with PROD and BROD enzyme activities, were evaluated at 3 and 7 days. These molecular markers were elevated in males and females exposed to 750 and 1500 ppm at both time periods in a phenobarbital-like manner. At 7 days, male rats exposed to 750 ppm sulfoxaflor and male and female rats at 1500 ppm sulfoxaflor showed significant hepatocellular proliferation; for males, it occurred in the centrilobular and midzonal regions, whereas for females, it was evident in the centrilobular region. Male rats exposed to 750 and 1500 ppm at both time periods and female rats exposed to 1500 ppm at day 7 showed significantly elevated levels of serum cholesterol; targeted gene expression analysis provided little insight into the possible MOA responsible for this increase (Table 38).

Cyp1a1 gene expression in male rats at 750 and 1500 ppm was slightly but significantly elevated at days 3 and 7; however, EROD enzyme activity was only slightly increased on day 3 and returned to control levels by day 7. Female rats showed a slight but significant increase in *Cyp1a1* gene expression at 1500 ppm on day 3, whereas no treatment-related changes in EROD levels were observed at either 3 or 7 days. Furthermore, gene expression of *Cyp4a22* was not elevated in this study. From these data, it is implied that sulfoxaflor is not likely an AhR or PPARα agonist.

Based upon these results, the MOA responsible for the increased liver weight in rats after administration of sulfoxaflor was phenobarbital-like, as evidenced by the CAR- and PXR-related molecular, enzymatic and proliferative responses. With the exception of a very mild induction of

Table 38. Summary of selected findings in a mechanistic study in rats

	Males				Females			
	Dietary concentration (ppm)							
	0	100	750	1500	0	100	750	1500
Body weight (g), day 3	177.8	167.4	168.0	164.9*	144.2	138.4	138.4	131.4*
Body weight gain (g), day 3	15.2	16.3	12.5	6.1	7.8	6.1	4.0	−0.1
Liver weight, absolute (g), day 3	7.47	7.24	7.78	7.84	5.18	5.09	4.95	4.87
Liver weight, relative (% of body weight), day 3	4.19	4.33	4.62	4.76*	3.59	3.68	3.58	3.71*
Body weight (g), day 7	198.7	209.3	195.2	184.6	147.3	150.1	147.8	144.7
Body weight gain (g), day 7	31.6	34.5	29.2	17.8	12.7	13.2	10.4	6.7
Liver weight, absolute (g), day 7	8.37	8.79	9.13	9.54	5.21	5.29	5.56	6.03
Liver weight, relative (% of body weight), day 7	4.20	4.18	4.67*	5.16*	3.54	3.53	3.76*	4.17*
Cholesterol (mg/dl), day 3	62	65	74*	80*	84	83	85	91
Cholesterol (mg/dl), day 7	60	61	80*	112*	75	76	82	106*
Targeted gene expression								
Cyp1a1, day 3	1	1.7	2.8	12.2	1	1.3	1.9	1.7*
Cyp2b1, day 3	1	9.7*	586*	1064*	1	3.6	399*	1204*
Cyp2b2, day 3	1	2.8*	8.2*	17.1*	1	2.5*	10.9*	21.4*
Cyp3a3, day 3	1	1.3	3.4*	8.8*	1	1.4	4.0*	7.8*
Alas1, day 3	1	−1.0	1.8*	2.4*	1	1.0	1.7*	2.6*
NADPH, day 3	1	1.1	2.0*	3.3*	1	1.1	1.7*	2.8*
Cyp1a1, day 7	1	1.1	3.2*	12.3*	1	−1.4	−1.0	2.4
Cyp2b1, day 7	1	7.2*	559*	848*	1	2.1	315*	856*
Cyp2b2, day 7	1	2.9*	10.2*	21.3*	1	1.5	6.7*	11.4*
Cyp3a3, day 7	1	1.2	3.3*	9.3*	1	−1.0	3.2*	6.6*
Alas1, day 7	1	1.2	1.6*	2.9*	1	1.0	1.8*	2.0*
NADPH, day 7	1	1.1	2.0*	3.2*	1	−1.0	1.6*	2.5*
Liver enzyme activity								
EROD (pmol/min per milligram protein), day 3	16.1	20.3*	25.9*	24.9*	26.6	25.3	24.9	28.8
PROD (pmol/min per milligram protein), day 3	2.9	2.7	24.4*	31.7*	3.7	3.3	18.3*	46.4*
BROD (pmol/min per milligram protein), day 3	2.8	3.5	6.4*	4.4*	3.2	3.1	16.4*	17.9*
EROD (pmol/min per milligram protein), day 7	16.3	18.0	18.2	16.3	31.4	30.9	31.7	26.7
PROD (pmol/min per milligram protein), day 7	3.5	4.6	33.9*	35.9*	4.7	5.3	61.4*	75.1*
BROD (pmol/min per milligram protein), day 7	3.5	4.6	16.9*	13.2*	2.6	3.1	27.6*	18.9*
Liver cell proliferation (Ki-67)								
Centrilobular (proliferation index), day 3	2.7	3.5	4.0	2.9	1.9	1.4	3.2	1.7
Midzonal (proliferation index), day 3	4.6	6.5	5.1	4.0	3.3	2.2	3.5	1.6
Centrilobular (proliferation index), day 7	3.3	4.1	7.8*	11.3*	1.7	1.3	2.6	4.5*
Midzonal (proliferation index), day 7	3.9	5.0	8.4*	11.2*	3.0	2.4	3.1	4.1

From Geter & Card (2010)
BROD, benzyloxyresorufin-*O*-debenzylase; EROD, 7-ethoxyresorufin-*O*-deethylase; PROD, 7-pentoxyresorufin-*O*-deethylase; * $P < 0.05$

Cyp2b1 and *Cyp2b2* gene expression in males, all other events occurred at concentrations above 100 ppm. These data support the conclusion that sulfoxaflor may be an agonist ligand primarily for CAR, and this activation results in the observed phenobarbital-like response and increased liver weight (Geter & Card, 2010).

(c) Mechanistic studies on Leydig cell tumour induction in rats

In a mechanistic study conducted to investigate the possible MOA responsible for the increase in bilateral Leydig cell tumour incidences in rats after long-term exposure to sulfoxaflor, groups of 15 male F344/DuCrl and 15 male Crl:CD(SD) rats were given diets containing sulfoxaflor (purity 95.6%) at a concentration of 0, 25, 100 or 500 ppm for up to 8 weeks. These dietary concentrations were equal to 0, 1.41, 5.58 and 27.8 mg/kg bw per day for F344/DuCrl rats and 0, 1.37, 5.59 and 27.7 mg/kg bw per day for Crl:CD(SD) rats. End-points evaluated included daily cage-side observations, weekly clinical observations and weekly examination of body weights and feed consumption. Also, in order to investigate enhanced biliary elimination of testosterone as a potential MOA, three rats per group underwent bile duct cannulation after 2 weeks of treatment to measure levels of [^{14}C]-testosterone-derived radioactivity within the bile and flow rate of the bile over a 2-hour period. In order to investigate dopamine agonism/enhancement as a potential MOA, a serum hormone panel of testosterone, luteinizing hormone (LH), prolactin and 17β-estradiol was evaluated on all available animals after 2, 4 and 8 weeks of treatment. In addition to hormone measurements, targeted gene expression analysis (quantitative RT-PCR using TaqMan gene expression assays) on LH and prolactin receptors in all Fischer 344 rat testes (4 and 8 weeks) as well as immunohistochemistry of LH receptors (LHR) in the testes of all necropsied rats at 4 weeks were performed. Quantification of LHR immunostaining was performed only on Fischer 344 rat testes (4 weeks only). During the 4- and 8-week necropsies, liver samples were collected for possible analysis; however, this was deemed not necessary based on results from the biliary elimination portion of the study indicating that the liver was not involved in the MOA. In addition, portions of testis were frozen during these necropsies as contingencies for immunohistochemistry on frozen sections, which was not necessary, as this was performed on formalin-fixed tissue.

There were no treatment-related clinical observations or effects on body weight or feed consumption in either strain during the study.

There was a treatment-related increase in absolute and/or relative liver weights of rats given 500 ppm sulfoxaflor in both strains at 4 weeks and in Fischer rats at 8 weeks (Table 39). There were no treatment-related effects on liver weights at 25 or 100 ppm or on testis weights at any dose level tested.

Quantification of LHR immunostaining on Fischer 344 rat testes performed at 4 weeks (the time point at which the largest hormone changes were observed) showed no treatment-related effect on the percentage of Leydig cells with cytoplasmic staining of LHR.

In bile duct–cannulated rats, there were no statistically significant or treatment-related differences in the mean [^{14}C]testosterone-derived radioactivity excreted in the bile across all dose groups, per time interval, for Crl:CD(SD) and F344/DuCrl rats. Bile flow was very similar for the respective dose groups, time intervals and strains. Overall, Crl:CD(SD) rats excreted approximately 1.5–3 times the cumulative amount of bile [^{14}C]testosterone-derived radioactivity compared with F344/DuCrl rats from the respective dose groups. The lower plasma radioactivity values for Crl:CD(SD) rats are consistent with having a higher biliary clearance than F344/DuCrl rats.

There was no effect of treatment on Fischer 344 rat serum hormone levels at the 2- or 8-week time points. After 4 weeks at 500 ppm, there was an approximate 2-fold increase in LH levels concomitant with an approximate 1.7-fold dose-dependent decrease in prolactin levels and an approximate 2.8-fold increase in testosterone levels. This hormone profile was somewhat recapitulated in Crl:CD(SD) rats, albeit with a different timing, where an increase in LH levels occurred with a

Table 39. Summary of selected findings in a mechanistic study on Leydig cell tumour induction in rats

	F344/DuCrl				Crl:CD(SD)			
	Dietary concentration (ppm)							
	0	25	100	500	0	25	100	500
Body weight (g), week 4	284	290	284	288	458	489*	457	481
Liver weight, absolute (g), week 4	10.2	11.0	11.0	12.4	17.6	19.7	17.8	21.0*
Liver weight, relative (% of body weight), week 4	3.6	3.8	3.9	4.3*	3.8	4.0	3.9	4.4*
Body weight (g), week 8	303	315	316	315	556	517	527	519
Liver weight, absolute (g), week 8	10.8	11.1	11.7	12.4*	20.7	18.9	19.3	19.9
Liver weight, relative (% of body weight), week 8	3.5	3.5	3.7	3.9*	3.7	3.7	3.7	3.8
Testes, LHR-positive cells (%), week 4	20.4	16.3	23.2	17.0	ND	ND	ND	ND
[^{14}C]Testosterone elimination in bile (%)	25.7	28.7	29.5	31.7	72.3	78.3	43.1	69.8
Serum hormone analysis								
Week 2								
Prolactin (ng/ml)	9.5	12.8	8.2	9.4	14.6	15.7	11.2	15.1
Testosterone (ng/g)	0.76	0.83	0.54	0.90	2.0	4.5*	3.7	3.7*
LH (ng/ml)	0.54	0.81	0.27	0.42	0.29	0.49	0.36	0.78*
Week 4								
Prolactin (ng/ml)	17.9	15.5	16.2	10.4	11.6	11.2	10.3	9.0
Testosterone (ng/g)	0.67	1.00	1.19	0.93	2.42	2.61	4.67	2.50
LH (ng/ml)	0.47	0.54	0.66	0.88*	1.10	0.34	0.36	0.35
Week 4, terminal								
Prolactin (ng/ml)	39.4	57.6	57.1	45.7	60.0	42.5	49.8	32.5
Testosterone (ng/g)	1.17	2.79	2.58	3.27	6.07	5.57	9.35	6.15
LH (ng/ml)	4.41	4.18	4.47	4.49	4.06	2.00	2.10	2.57
Week 8								
Prolactin (ng/ml)	19.3	17.9	19.5	17.5	14.5	16.5	24.3	19.1
Testosterone (ng/g)	0.58	0.67	0.77	0.70	1.75	2.74	2.43	1.94
LH (ng/ml)	0.89	1.04	0.69	1.08	0.25	0.47	0.66*	0.50
Week 8, terminal								
Prolactin (ng/ml)	70.9	60.5	53.7	71.9	35.3	34.0	41.0	33.2
Testosterone (ng/g)	2.56	2.42	2.56	2.68	3.01	3.41	4.28	3.79
LH (ng/ml)	4.37	4.64	3.84	4.75	1.86	1.71	2.50	2.32

From Rasoulpour et al. (2010d)
ND, not determined; * $P < 0.05$

concomitant increase in testosterone levels at 2 weeks, and a decrease in prolactin levels occurred at 4 weeks (Table 39).

Consistent with the decreased prolactin levels in the Fischer 344 rat at 4 weeks (500 ppm), there was a decrease in the LHR and the prolactin receptor gene expression at the 4-week, but not the 8-week, time point at 500 ppm (Table 40).

Table 40. Summary of targeted gene expression analysis in F344/DuCrl rats

	Fraction of control values			
	0 ppm	25 ppm	100 ppm	500 ppm
Week 4				
LHR	1	0.94	0.91	0.64
Prolactin receptor	1	0.79	0.97	0.62
StAR	1	0.80	1.16	0.96
Cyp11a1	1	0.97	1.24	0.87
Cyp17a1	1	0.53	0.91	0.44
HSD3b	1	0.78	1.04	0.73
SDR5a1	1	0.92	1.04	0.73
Week 8				
LHR	1	0.78	1.19	1.12
Prolactin receptor	1	0.79	1.33	1.08
StAR	1	0.79	1.22	1.14
Cyp11a1	1	0.84	1.57	1.45
Cyp17a1	1	0.96	2.12	1.34
HSD3B	1	0.76	1.36	1.06
SDR5a1	1	0.54	0.83	0.90

From Rasoulpour et al. (2010d)
Cyp11a1, P450 side-chain cleavage; *Cyp17a1*, 17α-hydroxylase; *HSD3b*, 3β-hydroxysteroid dehydrogenase; *SDR5a1*, steroid-5α-reductase; *StAR*, steroidogenic acute regulatory protein

There was no dose-dependent effect of treatment on any measured gene in the steroidogenic pathway, including *StAR* (steroidogenic acute regulatory protein), *Cyp11a1* (P450 side-chain cleavage), *Cyp17a1* (17α-hydroxylase), *HSD3b* (3β-hydroxysteroid dehydrogenase) or *SDR5a1* (steroid-5α-reductase) (Table 40).

The authors concluded that the study provides evidence supporting the hypothesis that the Leydig cell tumour promotion seen in the rat chronic toxicity and carcinogenicity study was through weak, but chronic, enhancement of dopamine release and subsequent inhibition of prolactin release from the pituitary gland, leading to increases in LH and testosterone levels, along with decreases in testis LHR gene expression. This MOA could operate through sulfoxaflor-mediated enhancement of dopamine release, potentially though agonism of α4β2 or α4α6β2 central nAChRs, which are known to play a key regulatory role in dopamine release from dopaminergic neurons in the brain (Rasoulpour et al., 2010d).

In a subsequent mechanistic study, sulfoxaflor (purity 95.6%) was evaluated for estrogen receptor (ER) alpha (ERα) and androgen receptor (AR) binding, ER and AR transactivation (agonism and antagonism) and aromatase inhibition. The ER and AR binding potential of sulfoxaflor was assessed in separate fluorescence polarization binding assays, which utilize a fluorescent ligand (Fluormone) and examine the ability of the test material to displace Fluormone from the receptor. The ERα used in these assays was a full-length receptor, whereas the AR consisted of only the ligand binding domain. The biological relevance of the ER and AR binding data was evaluated using a sensitive and reliable in vitro transactivation system. Specifically, the ability of sulfoxaflor was assessed for estrogen and androgen agonism and antagonism using stably transfected hERα-T47D-KBluc and hAR-MDA-kb2 cell lines, respectively. Aromatase inhibition of sulfoxaflor was evaluated by measuring the

conversion of an androgen to an estrogen using microsomes from cells expressing recombinant aromatase (CYP19). During the conversion of [^{3}H]androstenedione to estrone, ^3H_2O was released and quantified as a direct measurement of aromatase activity per unit reaction time. Competitive inhibition of aromatase activity by sulfoxaflor was then evaluated. A minimum of two independent runs of each assay were conducted for ERα and AR fluorescence polarization binding, ER and AR transactivation (agonism and antagonism) and aromatase activity. The duration of exposure was 24 hours for all transactivation assays. A complete concentration–response curve was run for each of the reference compounds and sulfoxaflor each time the transcriptional activation assay was performed. Aromatase inhibition assays were also run as two independent assays; androstenedione was used as the positive reference control.

Sulfoxaflor did not demonstrate any agonism or antagonism in the ER and AR transactivation assays. Reference controls demonstrated that the systems were performing as expected and able to detect mild agonism and antagonism for both ER and AR.

In two independent runs of the AR binding assay, sulfoxaflor exhibited a binding curve that passed 50%, which categorizes this test substance as a potential binder. However, the relative binding affinity of sulfoxaflor compared with dihydrotestosterone (DHT) was weak, with a mean relative binding affinity (compared with DHT) of 0.0014. Furthermore, the AR transactivation assays did not support the binding identified in the fluorescence polarization binding assay. As the AR in the fluorescence polarization binding assay included only the binding domain, it is possible that the binding that occurred in the fluorescence polarization assay could be an event that would not occur with the full-length AR receptor. Other mechanisms of action could also be causing the curve to cross 50% in the fluorescence polarization binding assay, including sulfoxaflor acting directly upon the receptor by denaturation.

In two independent runs of the ERα binding assay, sulfoxaflor did not displace Fluormone from the full-length ER, and no binding occurred. The ER transactivation assay supported the binding data, and sulfoxaflor was determined not to interfere with ER binding. Concurrent positive controls indicated specificity of the system to identify an ER binding compound.

In two independent runs of the aromatase assay, sulfoxaflor did not inhibit CYP19. Concurrent positive controls indicated the specificity of the system to identify an aromatase inhibitor.

Taken together, the results from these five different in vitro screening tests with sulfoxaflor did not indicate changes consistent with endocrine-mediated alterations (Toole, 2011).

A mechanistic study was performed in male Sprague-Dawley rats to determine the effect of sulfoxaflor (purity not stated), administered via intracerebral reverse microdialysis, on extracellular levels of dopamine and its two major metabolites, dihydroxyphenylacetic acid and homovanillic acid, in the hypothalamus. The experiment was performed the day following surgery. Dialysate samples were collected every 20 minutes from 80 minutes before drug administration until 280 minutes after drug administration (18 samples in total from each probe; 4 pre-drug and 14 post-drug). Treatments were administered to eight animals. Sulfoxaflor was infused directly into the hypothalamus at two concentrations (0.4 and 2 mmol/l) for 40 minutes each with the onset of the infusions 120 minutes apart (periods of infusion were 0–40 minutes and 120–160 minutes, respectively). Elevated potassium (50 mmol/l) was infused for 10 minutes to act as a positive control (240–250 minutes).

Sulfoxaflor at 0.4 mmol/l had no effect on the extracellular dopamine levels in the rat hypothalamus at any single time point; however, dopamine efflux averaged over the duration of the sulfoxaflor infusion was significantly ($P = 0.016$) increased. The higher concentration (2 mmol/l) resulted in a significant increase in extracellular dopamine, with a maximal rise of 39% ± 13% 40 minutes after the onset of infusion ($P < 0.001$), and dopamine efflux averaged over the duration of the sulfoxaflor infusion was also significantly ($P = 0.008$) increased. Infusion of potassium ions (50 mmol/l) increased dopamine levels, with a maximum increase of 78% ± 17% 20 minutes after onset of perfusion ($P < 0.001$), and

overall ($P < 0.001$). Both sulfoxaflor and potassium ions produced concomitant significant decreases in the concentration of homovanillic acid 40 minutes after the onset of infusion (18% ± 7%, $P < 0.05$; and 25% ± 6%, $P < 0.01$, respectively), whereas neither sulfoxaflor nor potassium ions altered the extracellular concentration of dihydroxyphenylacetic acid (Rowley & Heal, 2011).

(d) Mechanistic studies on fetal abnormalities and neonatal death in rats

A special study was conducted to examine the binding and the agonist activity of sulfoxaflor (purity 95.6%) on mammalian muscle nAChRs and to test the hypothesis that the high incidence of external limb abnormalities and misshapen clavicles is mediated by the pharmacological agonist action of sulfoxaflor at the fetal neuromuscular junction nAChR. This aim was achieved by use of competition radioligand binding and electrophysiological methods.

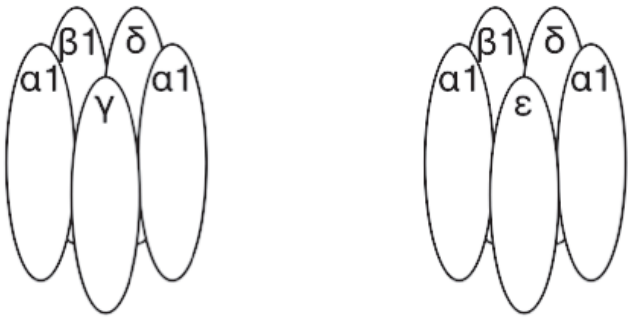
In both invertebrates and vertebrates, nAChRs comprise a diverse family of receptors, being assembled from a variety of distinct subunits. They are oligomeric cell surface proteins in which five subunits co-assemble in a doughnut-shaped arrangement. In the centre of the pentameric arrangement of subunits is a cation-selective ion channel. Binding of the endogenous neurotransmitter acetylcholine (ACh) (or of other agonists) stabilizes the open conformation of the ion channel and allows the influx of cations into the cell. In addition to nAChRs expressed in the mammalian central and peripheral nervous systems, nAChRs are also expressed at the neuromuscular junction. Five nAChR subunits are expressed in mammalian muscle cells (α1, β1, γ, δ and ε). Transcription of the γ and ε subunit genes is regulated developmentally, whereby the γ subunit is expressed in "fetal" muscle and the ε subunit is expressed in "adult" muscle. In rodents, it has been demonstrated that replacement of the γ subunit by the ε subunit initiates late during the 1st postnatal week and is largely complete by the end of the 2nd postnatal week, although in the extraocular muscles, expression of the γ subunit persists into adulthood. In humans, the switch from γ to ε subunit expression occurs predominantly during the late fetal period. Although many subtypes of nAChRs with distinct subunit composition have been identified in the central and peripheral nervous systems (neuronal nAChRs), only two nAChR subtypes are expressed in mammalian muscle (the fetal and adult muscle nAChRs). The subunit composition of the "fetal" and "adult" muscle nAChR can be represented as $(\alpha1)_2\beta1\gamma\delta$ and $(\alpha1)_2\beta1\delta\varepsilon$ (Figure 3).

Radioligand binding studies were performed to examine the ability of sulfoxaflor to bind to nAChRs from three mammalian species (human, rabbit, rat). Fetal muscle tissue was isolated from the rat and rabbit forelimb. Because of difficulty and concerns in obtaining and using human fetal muscle tissue, binding experiments with human nAChRs were performed with recombinant receptors, generated by expression in cultured human embryonic kidney cell lines of cloned complementary deoxyribonucleic acids (cDNAs) encoding human nAChR subunits for the "fetal" ($(\alpha1)_2\beta1\delta\gamma$) and "adult" ($(\alpha1)_2\beta1\delta\varepsilon$) muscle receptors.

The ability of sulfoxaflor to act as an agonist of nAChRs was examined using the technique of two-electrode voltage clamp recording, which allows the flow of current through cell surface nAChRs to be measured in response to agonist application. Receptors were expressed in *Xenopus* oocytes by microinjection of cDNA or complementary ribonucleic acid (cRNA) encoding the appropriate rat or human muscle nAChR subunits. To generate the fetal form of the muscle nAChRs, α1, β1, γ and δ cDNAs or cRNAs were injected, whereas α1, β1, δ and ε were injected to generate the adult form. With this expression system, the potency of agonist-induced nAChR activation can be measured in a quantitative manner, and evidence suggests that recombinant muscle nAChRs expressed in this system accurately mimic the pharmacological properties of native nAChRs. Functional responses (membrane currents) were detected in response to application of the endogenous agonist ACh.

The initial radioligand binding studies showed high levels of nonspecific binding of [^{3}H]sulfoxaflor. Therefore, competition binding was employed to examine whether unlabelled sulfoxaflor was able to displace binding of the high-affinity nAChR radioligand, [^{3}H]epibatidine. By fitting the concentrations of sulfoxaflor causing half-maximal displacement (median inhibitory concentrations

Figure 3. Subunit composition of the "fetal" and "adult" forms of the muscle nAChR



"Fetal" muscle nAChR **"Adult" muscle nAChR**

From Millar (2010)

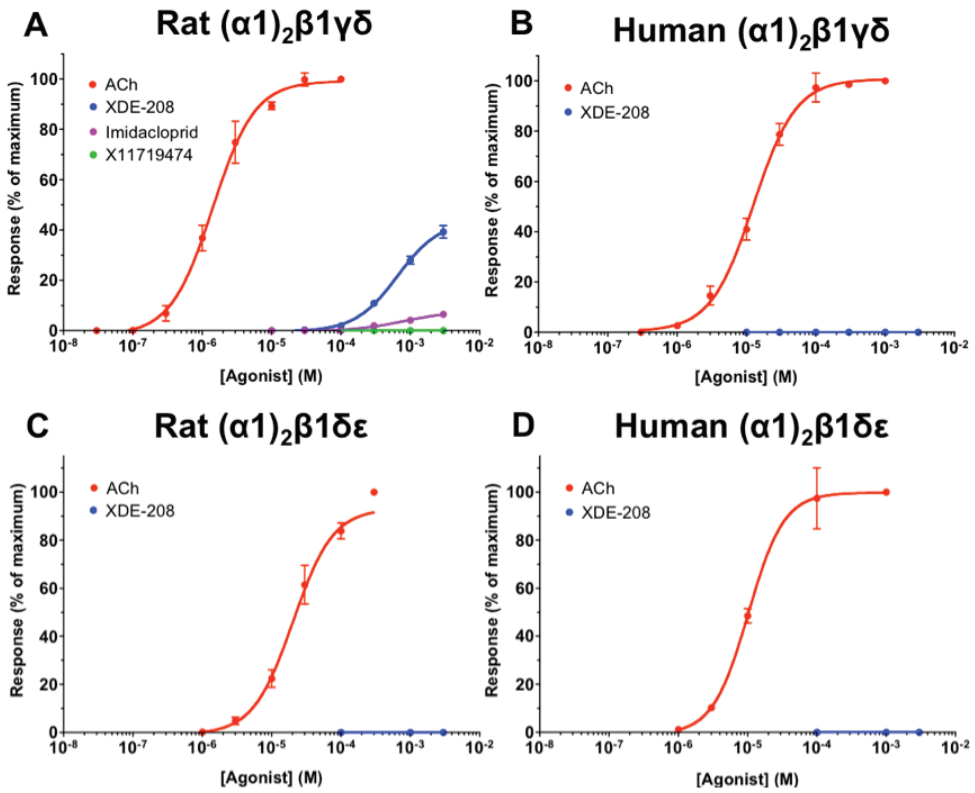
[IC_{50}s]) of 30 nmol/l [^{3}H]epibatidine to a single binding site model, estimates of IC_{50} for sulfoxaflor were 0.2 mmol/l for human, 0.4 mmol/l for rabbit and 2.3 mmol/l for rat. Although data obtained with human nAChRs and with rabbit muscle are fitted well by the single-site model, this was not the case for rat fetal muscle. Therefore, the binding data from rat muscle were fitted with a two-site model that revealed two distinct binding sites of different affinities (0.01 mmol/l and 8.9 mmol/l). The better fit of the rat fetal muscle nAChR experimental data to a two-site model would suggest that sulfoxaflor displaces [^{3}H]epibatidine from the two nAChR agonist binding sites (located at the α–γ and α–δ subunit interfaces) with different affinities.

The electrophysiological studies revealed that sulfoxaflor is a partial agonist of the rat fetal muscle nAChR. However, sulfoxaflor is a relatively low potency partial agonist, as the maximum response observed at the maximum feasible concentration (3 mmol/l, median effective concentration [EC_{50}] > 0.6 mmol/l) was 39% of that detected with a maximal concentration of ACh. In contrast, sulfoxaflor has no detectable agonist activity on the human fetal muscle nAChR or on the adult muscle nAChR from either human or rat (Figure 4). Imidacloprid, a neonicotinoid insecticide, also had partial agonist activity on the rat fetal muscle nAChR, but the maximal response detected with the highest concentration of imidacloprid tested (3 mmol/l) was approximately 7% of that detected with a maximal concentration of ACh and also only about 16% of the much lower maximal response detected with sulfoxaflor. X11719474, a soil metabolite of sulfoxaflor, had no significant agonist activity on the rat fetal muscle nAChR.

The results of this study show that sulfoxaflor is an agonist of the rat fetal muscle nAChR (which contains the rat γ subunit), whereas it has no agonist activity on the equivalent human fetal nAChR (containing the human γ subunit) or on the rat or human adult muscle nAChR (containing the rat or human ε subunit). It seems reasonable to conclude that the selective agonist activity of sulfoxaflor is due to differences in the amino acid sequence of the rat γ subunits compared with that of the human γ subunit (and also with the rat and human ε subunits) (Millar, 2010).

In a special study, a qualitative investigation of the action of sulfoxaflor on isolated phrenic nerve–hemidiaphragm preparations from newborn rats was conducted to assess the assumption that fetal limb contractions and reduced neonatal survival in rats following exposure during gestation result from the activation of the fetal muscle–type nAChR by sulfoxaflor, thereby causing sustained muscle contracture in the fetus and inhibition of nerve-evoked contraction of the diaphragm that would cause impaired respiration after birth, resulting in the previously observed reductions in neonatal survival. In support of this hypothesis, sulfoxaflor has been demonstrated to be an agonist at rat, but not human, embryonic ($(\alpha1)_2\beta1\delta\gamma$)) nAChR, while being without agonist activity at mature ($(\alpha1)_2\beta1\delta\varepsilon$)) muscle–type nAChRs (rat or human).

Figure 4. Agonist activation of nAChRs expressed in* Xenopus *oocytes. *Data are shown for the rat fetal $(\alpha1)_2\beta1\gamma\delta$ nAChR (A), human fetal $(\alpha1)_2\beta1\gamma\delta$ nAChR (B), rat adult $(\alpha1)_2\beta1\delta\varepsilon$ nAChR (C) and human adult $(\alpha1)_2\beta1\delta\varepsilon$ nAChR (D). AChRs were expressed by microinjection of cDNA or cRNA in* ***Xenopus*** *oocytes. Dose–response curves are shown in which agonist-evoked responses are normalized to the maximal response detected with the endogenous agonist, ACh. XDE-208, sulfoxaflor; X11719474, metabolite of sulfoxaflor. Data points are means ± standard error of the mean of 3–7 responses.*



From Millar (2010)

Isolated phrenic nerve–hemidiaphragm preparations from newborn (PND 0) Sprague-Dawley rats (supplier, UCL Biological Services) were used. Recorded measures included 1) changes in muscle twitch and 2) muscle contracture following test material application to the bath perfusion system. Muscle twitch tension reflects phrenic nerve action potential-evoked brief contraction of the diaphragm and is displayed as an upward deflection of the recording trace. Muscle contracture reflects a test material–induced prolonged contraction of the diaphragm associated with increased muscle tension beyond that evoked via electrical stimulation of the phrenic nerve. In each experiment, a period of at least 3 minutes of stable baseline and twitch tension was recorded before application of any test material. Preparations were viable for between 1 and 3 hours and could respond repeatedly to test material application.

Sulfoxaflor at a high concentration (1 mmol/l) consistently produced a reversible, concentration-dependent contracture of the neonatal diaphragm muscle and a decrease in muscle twitch response similar in magnitude to that observed with ACh (100 µmol/l). The contracture was blocked by the selective muscle-type nAChR antagonist, tubocurarine (10 µmol/l), showing that the contracture induced by sulfoxaflor is mediated via nAChR activation, rather than via a post-receptor mechanism. Furthermore, prolonged application of sulfoxaflor caused a sustained muscle contracture. Muscle twitches in response to phrenic nerve stimulation were not affected at low sulfoxaflor

concentration (100 µmol/l) but were reduced at high concentration (1 mmol/l), demonstrating that sulfoxaflor can cause inhibition of nerve-evoked contraction of the diaphragm during sustained contracture, consistent with the observed impairment of respiration in the neonatal rat.

The results of this study demonstrate that sulfoxaflor caused a contracture of the newborn rat diaphragm by acting on the nAChR. Prolonged application caused a sustained muscle contracture and a contracture-associated inhibition of the phrenic nerve–evoked muscle twitch, which is considered analogous to the situation in vivo, which resulted in fetal limb contractions (sustained muscle contractions) and compromised respiration at birth (contracture-associated inhibition of the muscle twitch). The results are consistent with, and add additional support to, the hypothesis that sulfoxaflor causes neonatal death (and fetal abnormalities) via activation of the fetal muscle–type nAChR (Gibb, 2010).

A special histopathological evaluation of fetal lung samples from the prenatal developmental toxicity study in rats (Rasoulpour, Marshall & Saghir, 2010) was conducted to detect any morphological abnormalities (e.g. increased collagen deposition) in any region of the lungs that may have been contributory to the treatment-related increase in neonatal pup mortality. Samples were collected from two formalin-fixed fetuses (one male and one female) per dam from five control and four 1000 ppm litters (18 samples total). Trachea and lungs of the selected fetuses were routinely processed for histology; sections were cut at a thickness of 5–6 µm, stained with haematoxylin and eosin, and examined.

There were no sulfoxaflor-induced lesions in the trachea, bronchi, bronchioles or alveoli in any of the treated fetuses examined. There were no treatment-related increases in collagen deposition around the airways or alveolar walls or any other changes. All observations were considered within normal limits. Thus, histopathological examination of the trachea and lungs of selected fetuses from dams given 1000 ppm sulfoxaflor from GD 6 to GD 21 did not reveal any morphological abnormalities that could have contributed to the sulfoxaflor-induced neonatal mortality in rat pups (Thomas & Marshall, 2010).

(e) Studies with metabolites

The structures of the sulfoxaflor metabolites tested in the toxicological studies described below are given in Table 41.

Studies with X11719474 (soil and plant metabolite of sulfoxaflor)

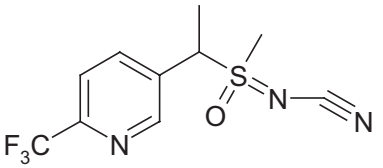
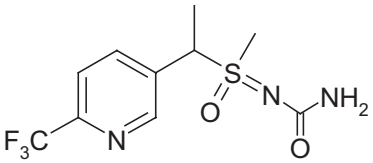
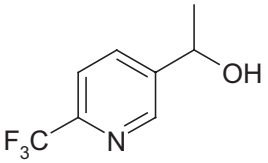
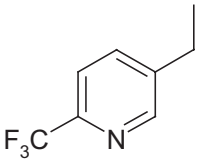
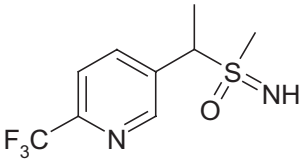
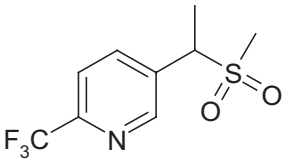
In a non-guideline pilot study to determine toxicokinetic properties, groups of one male and one female (F344/DrCrl) rat were treated with X11719474 (radiochemical purity 98.8%, specific activity 1.73 GBq/mmol) formulated in 0.5% aqueous methylcellulose at 100 mg/kg bw by oral gavage. Toxicokinetics, mass balance and urinary metabolite profile were determined in animals of one group, and the profile of plasma metabolites was determined in a second group.

The test compound was rapidly absorbed upon oral administration. Absorption was almost complete (95%/98% in males/females, respectively). Total recovery was 98%/99% (males/females). The majority of the administered dose (91–95%) was recovered in urine within 12 hours post-administration and increased slightly until 168 hours post-dosing. Low amounts (2–3%) were excreted via faeces over the total observation period. Plasma elimination of test compound was biphasic, with a rapid initial phase ($t_{½} \leq 2$ hours) and a slower second phase ($t_{½} \leq 36$–41 hours). Red blood cell elimination was monophasic and slightly slower than that from plasma.

Absorbed material was essentially unmetabolized in both sexes. In urine, mainly parent compound and two minor (< 1%) metabolites or impurities were detected (Hansen et al., 2010).

In a non-guideline, non-GLP acute oral toxicity study, three female (F344/DuCrl) rats received X11719474 (purity 100%) in 0.5% aqueous methylcellulose at a single dose of 300 mg/kg bw by

Table 41. Structures of sulfoxaflor metabolites tested in toxicological studies

Substance	IUPAC chemical name	Structure
Sulfoxaflor	[Methyl(oxo){1-[6-(trifluoromethyl)-3-pyridyl]ethyl}-λ⁴-sulfanylidene]cyanamide	
X11719474 (soil and plant metabolite)	*N*-(Methyl(oxido){1-[6-(trifluoromethyl)pyridin-3-yl]ethyl}-λ⁴-sulfanylidene)urea	
X11721061 (plant and animal metabolite)	1-[6-(Trifluoromethyl)pyridin-3-yl]ethanol	
X11596066 (animal metabolite)	5-Ethyl-2-trifluoromethylpyridine	
X11579457 (soil metabolite)	5-[1-(*S*-Methylsulfonimidoyl)ethyl]-2-(trifluoromethyl)pyridine	
X11519540 (soil and animal metabolite)	5-[(1-Methylsulfonyl)ethyl]-2-(trifluoromethyl)pyridine	

gavage at a volume of 10 ml/kg bw. The rats were monitored daily for mortality and clinical signs for a 72-hour period. Animals were not examined for gross pathological changes.

None of the animals died or showed any signs of toxicity. Under the conditions of the study, the estimated acute oral LD_{50} in rats was above 300 mg/kg bw in females (Golden, 2007/2010).

In an acute oral toxicity study conducted according to OECD test guideline 423 (“acute toxic class method”), female Fischer 344 rats received X11719474 (purity 99%) in distilled water at a single dose of 2000 (one animal) or 5000 mg/kg bw (three animals) by gavage at a volume of 24.8 ml/kg bw (because of the high volume, the high-dose group received the dose split into two approximately equal portions). The rats were monitored daily for mortality and clinical signs for a 2-week period, and body weights were recorded on days 1, 7 and 14. On day 14, surviving animals were killed, necropsied and examined for gross pathological changes.

The female dosed with 2000 mg/kg bw and the three females dosed with 5000 mg/kg bw survived. In the high-dose animals, clinical signs following administration included mouth and nasal discharge and/or anogenital staining. Findings regarding detailed clinical observation were noted in mucous membranes, salivation, locomotion, posture, piloerection and/or defecation for all three rats between 3 hours and 2 days post-dosing. However, all animals recovered from the above signs by day 3 and appeared active and healthy for the remainder of the 14-day observation period. No gross abnormalities were observed in any treated animals. The estimated acute oral LD_{50} in female rats was greater than 5000 mg/kg bw (Durando, 2010a).

In a non-guideline, non-GLP acute dermal toxicity/skin irritation screening study, three female F344/DuCrl rats received X11719474 (purity 100%) moistened with 0.5% aqueous methylcellulose at a single dose of 1000 mg/kg bw. The test compound was applied to the back of the animals (which was clipped free of hair on the day before treatment), covered with a gauze patch and wrap and held in place with elastic tape. After 24 hours, the test compound was removed. The rats were monitored daily for mortality and clinical signs for a 72-hour period, and the application site was graded for erythema and oedema within 60 minutes and 24, 48 and 72 hours after removal of the test material. Animals were not examined for gross pathological changes.

None of the animals died. Body weight loss was observed, which was attributed by the study authors to stress caused by semi-occlusive wrapping. Sixty minutes after removal of the test compound, very slight erythema was seen in two animals, and well-defined erythema and slight oedema were observed in the remaining animal. At later skin readings, the skin appeared normal. Under the conditions of the study, the estimated acute dermal LD_{50} in rats was above 1000 mg/kg bw in females. The test compound induced slight dermal irritation, which resolved within 24 hours after removal of the compound (Brooks & Golden, 2008/2010).

In a non-guideline, non-GLP eye irritation screening study, one female New Zealand White rabbit received X11719474 (purity 100%) in an amount of 0.1 ml (69 mg) in the lower conjunctival sac of the right eye. The left eye served as untreated control. Owing to discomfort after dosing, both eyes received local anaesthetic. Eyes were examined for conjunctival redness, chemosis, discharge, corneal opacity and iris irritation at 1, 24, 48 and 72 hours post-dosing.

Slight redness, chemosis and discharge were observed in the treated eye 1 hour after the administration. These findings had resolved by the next reading (24 hours) (Brooks & Golden, 2008/2010).

In a non-GLP reduced local lymph node assay conducted according to OECD test guideline 429 (2002), six female CBA/J mice received X11719474 (purity 100%) dissolved in DMSO at a concentration of 50%. The test compound was applied to both ears in a volume of 25 µl/ear on 3 consecutive days. The dose level was chosen on the basis of preliminary results showing that concentrations of 5%, 25% and 50% caused no effects on body weight and did not induce erythema. An additional group received the vehicle (DMSO) to serve as negative control. Animals were checked for mortality and clinical signs at least daily during the study. In particular, the site of application was examined for signs of local irritation. Individual body weights were measured at study start and at scheduled termination.

No mortality or clinical signs were observed during the study. In particular, no cutaneous reactions were observed at the application site of animals treated with the test compound. Body weight changes were comparable between control and treated groups. A negative response was observed at the tested dose level (stimulation index: 1.3). No concurrent positive control group was included in the study (according to the study result, a previous study with hexylcinnamaldehyde showed the expected result; however, no details on the results were given). On the basis of this study, X11719474 did not show any sensitization potential (Wiescinski & Sosinski, 2008/2010).

In a reverse gene mutation assay in bacteria conducted according to OECD test guideline 471 (1997), *Salmonella typhimurium* (strains TA98, TA100, TA1535, TA1537) and *Escherichia coli* (strain WP2*uvr*A) were exposed to X11721061 (purity 99%), using DMSO as solvent, in the presence and absence of S9 metabolic activation in two independent sets of experiments. Doses were selected based on the guideline limit dose and results of a range-finding experiment. For the main preincubation test using doses of up to and including 5000 µg/plate, three plates were used for each strain, condition and dose. Vehicle and positive controls were included in each experiment. The independent repeat test utilized the same conditions as the initial test. Doses up to and including 5000 µg/plate did not cause any bacteriotoxic effects. Total bacteria counts remained unchanged, and no inhibition of growth was observed.

No evidence for mutagenic activity of X11721061 was seen. No biologically relevant increase in the mutant count, in comparison with the negative controls, was observed. The positive controls induced the appropriate responses in the corresponding strains. Therefore, X11721061 was considered to be not mutagenic in the bacterial strains tested, in either the presence or absence of metabolic activation (Mecchi, 2008).

In an in vitro mammalian cell gene mutation test conducted according to OECD test guideline 476 (1997), X11719474 (purity 100%) dissolved in DMSO was tested for its ability to induce forward mutations at the *HPRT* locus in CHO cells. Two independent sets of experiments were conducted in the presence and absence of S9 metabolic activation. Based on the guideline limit dose (10 mmol/l), concentrations of 184.6–2953 µg/ml were used in the main study both with and without metabolic activation, and the same concentrations were used for the independent repeats. EMS and 20-MCA served as positive controls in the experiments without and with metabolic activation, respectively. The cells were treated for 4 hours in both experiments, without and with metabolic activation. After this, the incubation media were replaced by culture medium, and the cells were incubated (and subcultured every 2–3 days) for about 7–9 days for expression of mutant cells. This was followed by incubation of cells for 6–10 days in selection medium containing 6-thioguanine.

No increase in mutant frequency was observed in either the initial or the confirmatory study. In contrast to this, the positive control substances EMS and 20-MCA resulted in a marked increase in mutant frequency. Based on the results of the study, X11719474 was considered to be not mutagenic in the CHO/HPRT forward mutation assay, in either the presence or absence of metabolic activation (Schisler & Geter, 2008).

In an in vitro mammalian chromosomal aberration test conducted according to OECD test guideline 473 (1997), the clastogenic potential of X11719474 (purity 100%) dissolved in DMSO was tested in rat lymphocytes (whole blood). Based on the results of the mitotic index, concentrations of 369.1, 738.25 and 2953 µg/ml were used with metabolic activation and 738.25, 1476.5 and 2953 µg/ml were used without metabolic activation in an experiment with 4 hours of treatment and harvest at 24 hours (i.e. 20 hours post-exposure). In addition, with 24 hours of treatment without S9 mix, concentrations of 738.25, 1476.5 and 2953 µg/ml were chosen. Vehicle (DMSO) and positive controls (cyclophosphamide and mitomycin C for the test with and without metabolic activation, respectively) were included to demonstrate the sensitivity of the test system. Cultures were set up in duplicate. After slide preparation and staining of the cells, 200 metaphases per dose and treatment condition were analysed for chromosomal aberrations.

None of the cultures treated with X11719474 in the absence or in the presence of S9 mix showed any biologically relevant or statistically significant increase in the numbers of aberrant metaphases. No change in the frequency of polyploid metaphases was observed. The positive controls mitomycin C and cyclophosphamide induced clastogenic effects and demonstrated the sensitivity of the test system and the activity of the S9 mix used. Based on the results of this test, X11719474 was considered

not to be clastogenic for mammalian cells in vitro, in either the presence or absence of metabolic activation (Schisler, Kleinert & Geter, 2008).

In a non-guideline, non-GLP palatability study, groups of three male and three female Fischer 344 rats were fed diets containing X11719474 (purity 99.6%) at a dose of approximately 0, 125, 250, 500 or 1000 mg/kg bw per day for up to 7 days to evaluate palatability, general toxicity and toxicokinetics. Parameters evaluated were daily cage-side observations, body weights, feed consumption, clinical pathology and gross examinations. Liver weight was recorded, and liver sections underwent histopathological examination. Urine and blood plasma were analysed for toxicokinetics.

No mortalities or abnormal clinical observations were found during the treatment period in any treatment group. Animals treated with 1000 or 500 mg/kg bw per day showed low feed intake and body weight and body weight gain. These body weight effects remained for the whole study period in the high-dose groups and in the 500 mg/kg bw per day females group and were reversible by the end of the treatment period in the 500 mg/kg bw per day male group. Feed intake was altered only during the first study days.

The levels of the test compound in blood plasma or in urine increased nearly linearly with the test material intake. Triglyceride levels were lower in 1000 and 500 mg/kg bw per day dose group males and females. Other clinical pathology parameters showed no biologically relevant changes.

No gross abnormalities were observed. Absolute and relative liver weights were increased in males (approximately 15% or 28% increase in relative weight) and females (approximately 9% or 13% increase in relative weight) of the 500 and 1000 mg/kg bw per day dose groups. In sections of liver, midzonal to centrilobular hepatocyte hypertrophy and altered tinctorial properties (cytoplasmic eosinophilia) were observed in 500 and 1000 mg/kg bw per day dose group males (very slight to slight) and in 1000 mg/kg bw per day dose group females (very slight).

Therefore, under the conditions of this study, X11719474 was sufficiently palatable at concentrations up to approximately 500 mg/kg bw per day for studies of repeated administration (Yano et al., 2010a).

In a toxicity study conducted according to OECD test guideline 407 (1995), groups of five male and five female Fischer 344 rats were given diets containing X11719474 (purity 99.5%) at a concentration of 0, 1000, 2000, 3000 or 8000 ppm (equal to 0, 83.4, 167, 244 and 662 mg/kg bw per day in males and 0, 90.1, 184, 278 and 734 mg/kg bw per day in females) for 28 days. Animals were observed daily for mortality and clinical signs. Physical examinations were performed weekly. Body weight and feed consumption were recorded once weekly. During the acclimatization phase, all animals were subjected to an ophthalmic examination; all animals were re-examined prior to scheduled necropsy. Haematology, clinical chemistry and urine parameters were determined at the end of the study. All animals were necropsied, selected organs were weighed and a range of tissues were taken, fixed and examined microscopically. Bone marrow of one femur of each animal was collected, and slides were prepared and archived, but not evaluated for the induction of micronuclei (five males treated with a positive control substance were included, but no details on the compound or the dose level were included in the report). A liver piece from each animal was stored for possible later gene expression analysis.

No dose-related effect on mortality rate was reported. There were no treatment-related effects on clinical signs, ophthalmic observations, or haematological or coagulation parameters. Males and females given 8000 ppm exhibited reduced body weight gain and reduced feed intake compared with controls during the first 5 days; however, body weight and feed intake were comparable to those of controls towards the end of the treatment period. Uric acid crystals were detected in urine of males and females of the top-dose group. Additionally, amorphous urate crystals were noted in top-dose females.

Blood cholesterol levels for males and females given 8000 ppm were higher than those of the controls. Males and females given 8000 ppm had higher absolute (33% and 14%, respectively) and relative (37% and 18%, respectively) liver weights than the controls. Males and females given 8000 ppm and males given 3000 ppm had a slight treatment-related hypertrophy of hepatocytes (with increased cytoplasmic eosinophilia) in the centrilobular/midzonal region of the hepatic lobules. All other organ weight changes and histopathological findings were considered non-adverse.

Absorption of X11719474 from the diet was dose proportional for both sexes, based on plasma concentrations of parent compound. The ingested X11719474 was rapidly eliminated from plasma upon removal of the fortified diet (fasting) and dropped below the limit of quantification at the time of sacrifice in most of the rats. The elimination half-life of X11719474 from plasma of rats with detectable parent test material was between 2.7 and 3.6 hours. Male rats eliminated less of what they consumed during a 24-hour period, when compared with females. Overall, these data show that X11719474 was significantly absorbed following dietary administration and quickly eliminated, primarily via urine, as parent compound. The ratio of diastereomers was not substantially different in the plasma and urine samples when compared with the matrix samples added with the test material.

The NOAEL was 3000 ppm (equal to 244 mg/kg bw per day in males and 278 mg/kg bw per day in females), based on initially reduced body weight gain and feed consumption, liver findings (increased plasma cholesterol levels, organ weight increase) and urinary crystals in males and females at 8000 ppm (Yano et al., 2010b).

In a toxicity study conducted according to OECD test guideline 408 (1998), groups of 10 male and 10 female Fischer 344 rats were given diets containing X11719474 (purity 99.5%) at a concentration of 0, 500, 1000 or 5000 ppm (equal to 0, 32.2, 65.3 and 327 mg/kg bw per day in males and 0, 35.2, 71.8 and 352 mg/kg bw per day in females) for at least 90 days. Animals were observed daily for mortality and clinical signs. Physical examinations were performed weekly. In addition, neurological/functional tests were conducted once during the acclimatization phase and during the last week of the study. Body weight and feed consumption were recorded once weekly. During the acclimatization phase, all animals were subjected to an ophthalmic examination; all animals in the control and treatment groups were re-examined prior to scheduled necropsy. Haematology, clinical chemistry and urine parameters were determined at the end of the study. All animals were necropsied, selected organs were weighed and a range of tissues were taken, fixed and examined microscopically. A liver piece from each animal was stored for possible later gene expression analysis. Compound concentrations (and known major metabolites) were analysed in blood plasma and urine in the last week of treatment for toxicokinetic analysis.

Four days prior to scheduled necropsy, animals were immunized with sheep red blood cells by intravenous injection to determine possible treatment-related effects on the immune system. An additional five animals per sex were treated with cyclophosphamide on the 4 days prior to scheduled necropsy to serve as a positive control for the determination of immunotoxic effects.

No treatment-related effects on mortality, clinical signs, functional tests, body weights, feed intake, ophthalmoscopic examinations, haematology, urinalysis or immune system were detected.

Male and female animals in the top-dose group had higher plasma cholesterol levels (approximately 18–19%) compared with control animals. Additionally, males in the top-dose group had increased relative and absolute liver weights (approximately 16% or approximately 17%, respectively). Livers of top-dose animals showed slight treatment-related hypertrophy of hepatocytes (with increased cytoplasmic eosinophilia) in the centrilobular/midzonal region of the hepatic lobules. Males were affected more severely and also showed very slight, multifocal, individual cell necrosis of centrilobular hepatocytes and vacuolation of hepatocytes, consistent with fatty changes. All other organ weight changes (thyroid and adrenals in males) were considered non-adverse.

Toxicokinetic analysis of plasma after 13 weeks of treatment indicated a dose-proportional systemic exposure across all dose groups in both sexes. The plasma elimination half-life was approximately 7.8 or 7.5 hours in mid-dose animals and 5.2 or 4.1 hours in high-dose males or females, respectively. The test compound was almost completely absorbed and eliminated via urine as unchanged compound.

The NOAEL was 1000 ppm (equal to 65.3 mg/kg bw per day in males and 71.8 mg/kg bw per day in females), based on liver toxicity in both sexes at 5000 ppm (equal to 327 mg/kg bw per day in males and 352 mg/kg bw per day in females) (Yano et al., 2010c).

In a non-guideline palatability study, groups of two male Beagle dogs were administered X11719474 at a dose of 50 or 100 mg/kg bw per day by gavage (10 ml/kg bw per day, formulated in 0.5% aqueous methylcellulose) for up to 7 days to evaluate palatability and general toxicity. Parameters evaluated were daily cage-side observations, body weights and feed consumption.No further examinations of animals after euthanization at study termination were performed.

Oral administration of the test compound at dose levels of 50 or 100 mg/kg bw per day for 7 days was well tolerated by the animals. No adverse effects on body weight or feed intake were observed. One of two males treated with 100 mg/kg bw per day exhibited faecal changes (soft, mucoid and/or discoloured) during the dosing period (Heward, 2009/2010).

In a toxicity study conducted according to OECD test guideline 409, groups of four male Beagle dogs were administered X11719474 (purity 99.5%) by oral gavage in 0.5% aqueous methylcellulose (Methocel A4C) at a dose of 0, 10, 25 or 50 mg/kg bw per day for 90 days. The dose volume was 10 ml/kg bw. The study was intended to compare the properties of the metabolite X11719474 with sulfoxaflor in the available 90-day toxicity study (Stewart, 2010) using as few animals as possible (i.e. only animals of one sex). Hence, the dose levels were selected based on the results of the toxicity study in dogs conducted with sulfoxaflor (LOAEL 10 mg/kg bw per day, NOAEL 6 mg/kg bw per day) and 5-fold and 10-fold increases over the NOAEL. The results in rats (Yano et al., 2009c, 2010c) supported the assumption that the metabolite was less toxic than the parent compound.

Observations for morbidity, mortality, injury and the availability of feed and water were conducted twice daily for all animals. Clinical observations were conducted, and body weights were measured and recorded weekly. Feed consumption was measured weekly. Physical examinations were conducted by a veterinarian pretest to confirm the good health status of each animal placed on study. Blood and urine samples for clinical pathology evaluations were collected from all animals pretest and during weeks 6 and 13. Blood and urine samples were collected from all animals at designated intervals during week 13 for determination of the plasma concentrations of the test material (toxicokinetics). At study termination, necropsy examinations were performed, organ weights were recorded and selected tissues were examined microscopically.

There were no mortalities and no treatment-related clinical observation findings at any dose level. No treatment-related effects were noted on body weight, feed intake, clinical pathology or urinalysis parameters in all dose groups.

There were no treatment-related gross pathological observations, organ weight changes or histopathological changes at any exposure level.

The toxicokinetic analysis after 13 weeks of dosing at 10, 25 or 50 mg/kg bw per day showed that the daily systemic dose ($AUC_{24\ h}$) was 126.2 ± 9.5, 298.4 ± 47.9 and 682.3 ± 82.9 µg·h/ml in males, respectively. The increase in systemic dose was clearly dose proportional in male dogs across all three dose levels. The mean plasma elimination half-life of the test compound at 10, 25 or 50 mg/kg bw per day was 8.4 ± 2.4, 7.8 ± 1.0 and 7.7 ± 1.1 hours, respectively.

The NOAEL was greater than 50 mg/kg bw per day, the highest dose tested (Heward, 2010b).

In a reproduction/developmental toxicity screening study conducted according to OECD test guideline 421, groups of 12 male and 12 female Crl:CD(SD) rats were given diets containing X11719474 (purity 99.5%) at a concentration of 0, 1000, 2000 or 5000 ppm (equal to 0, 80.8, 162 and 396 mg/kg bw per day in males and 0, 81.7, 167 and 451 mg/kg bw per day in females) (pre-mating phase), respectively. Males were fed the test diets for 2 weeks prior to breeding and continuing throughout breeding until termination. The females were fed the test diets for 2 weeks prior to breeding, continuing through breeding (up to 2 weeks), gestation, lactation and weaning; pups were weaned on PND 21. Effects on gonadal function, mating behaviour, conception, development of the conceptus, parturition and postnatal growth and survival were evaluated. In addition, a gross necropsy and histopathological examination of the adults were conducted with an emphasis on organs of the reproductive system. In the offspring, litter size, pup survival, sex, body weight and the presence of gross external morphological alterations were assessed.

All parental animals survived until termination, and there were no treatment-related clinical observations or changes in feed intake at any dose level throughout the study. Females in the 2000 and 5000 ppm dose groups had decreases in body weight and/or body weight gain during isolated gestational and lactational intervals; however, these were considered to be non-adverse.

Males and females of the 5000 ppm dose groups had increased absolute and relative liver weights that were treatment related. Treatment-related histological effects were observed in the livers of males and females given 5000 ppm and consisted of very slight hepatocellular hypertrophy, with altered tinctorial properties, involving the centrilobular to midzonal regions of the hepatic lobule.

There were no reproductive or developmental toxicity effects observed in any group up to PND 21. There were no effects on pup body weight in any dose group.

Toxicokinetic analyses of plasma samples from PND 4 culled pups indicated a dose-proportionate increase in the systemic concentration of the test compound.

The NOAEL for parental toxicity was 2000 ppm (equal to 162 mg/kg bw per day), based on slight signs of liver toxicity. The NOAEL for offspring and reproductive performance was 5000 ppm (equal to 396 mg/kg bw per day), the highest dose tested (Rasoulpour et al., 2010a).

In a prenatal developmental toxicity study conducted according to OECD test guideline 414, groups of 26 time-mated female Crl:CD(SD) rats were given diets containing X11719474 (purity 99.5%) at a concentration of 0, 1000, 2000 or 5000 ppm (equal to 0, 74.4, 152 and 368 mg/kg bw per day) on GDs 6 through 21. In-life maternal study parameters included clinical observations, body weight, body weight gain and feed consumption. On GD 21, all rats were euthanized, and all dams and fetuses were examined for gross pathological alterations. In addition, blood was collected from dams and fetuses to determine blood levels of the test material. Liver, kidneys and gravid uterine weights were recorded, along with the numbers of corpora lutea, uterine implantations, resorptions and live/dead fetuses. All fetuses were weighed, sexed and examined for external alterations. Approximately one half of the fetuses were examined for visceral and craniofacial alterations, whereas skeletal examinations were conducted on the remaining fetuses.

Maternal toxicity at 5000 ppm was limited to transient decreases in body weight gain at the initiation of treatment, relative to controls, with concomitant decreased feed consumption. As a result of the low extent and limited duration, these findings were considered to be non-adverse by the study director.

No dose-related effects on reproductive parameters, gross pathology or organ weights were reported in any dose group.

The external and visceral examination of fetuses revealed no treatment-related effects on development of offspring. In the 5000 ppm dose group, three fetuses from two litters had class I wavy ribs (variation), and two fetuses from two litters had class II wavy ribs (malformation). The former was

within the historical control range, whereas the latter was slightly outside the historical control range. Additionally, slight incidences of calloused ribs and delayed ossification of parietal, interparietal or occipital bones were observed in high-dose fetuses. These findings were within the historical control range. The study director stated that "the incidence of class II wavy ribs was slightly outside historical control range, these findings occurred in foetuses with other rib alterations and occurred at very low incidences therefore they were considered spurious and unrelated to treatment".

The terminal plasma concentrations of test compound in fetal blood were dose proportional throughout the entire range of dietary exposure concentrations.

The NOAEL for maternal toxicity and for prenatal developmental was 5000 ppm (equal to 368 mg/kg bw per day), the highest dose tested (Rasoulpour & Marshall, 2010).

In a non-GLP, non-guideline study, six male F344/DuCrl rats were given test diets formulated to supply 0 or 8000 ppm X11719474 in the diet for 7 days, which corresponded to time-weighted average doses of 0 or 583 mg/kg bw per day, respectively. The purpose of this study was to generate information to aid in understanding the MOA for liver effects in rats induced by X11719474 and to compare the results with those observed following exposure to sulfoxaflor (1500 ppm, equal to 102 mg/kg bw per day; Geter & Card, 2010, see above). The treatment level of 8000 ppm was the high dose level used in the rat 28-day toxicity study. The parameters evaluated were daily cage-side observations, body weight, body weight gain, feed consumption, liver weights, histopathology of the liver, focused gene expression, liver cytochrome P450 enzyme activity and hepatocellular proliferation using BrdU immunohistochemical methods.

At the end of the 7-day treatment period, males exposed to 8000 ppm X11719474 had reduced body weight and body weight gain of approximately 2% and 22% relative to controls, respectively. In addition, males given 8000 ppm X11719474 had increases in liver weights (absolute 16%; relative 19%). X11719474 induced liver weight increases that correlated with treatment-related microscopic observations of very slight centrilobular/midzonal hypertrophy of hepatocytes that were present in all compound-treated animals. At the end of the 7-day treatment period, males exposed to 8000 ppm X11719474 had the same types of treatment-related effects as were observed with the parent compound, but were consistently less marked, despite the almost 6-fold higher dose level.

The CAR-related transcripts, *Cyp2b1* and *Cyp2b2*, along with the PXR-related transcript, *Cyp3a3*, were increased 806-, 32- and 4-fold, respectively. Parent compound–induced gene expression showed a similar profile of 848-, 21- and 9-fold, respectively. The level of hepatocellular PROD enzyme activity, a CAR-associated biomarker, was increased 15-fold following X11719474 exposure. A similar level of induction (10-fold) was seen for parent compound–induced PROD enzyme activity. Hepatocellular proliferation, as measured by BrdU incorporation, was significantly elevated by 4.5-, 2.5- and 2.1-fold in the centrilobular, midzonal and periportal regions, respectively. A hepatocellular proliferation study with parent compound (Geter & Card, 2010), as measured by Ki-67, showed increased proliferation in the centrilobular and midzonal regions of 3.4- and 2.9-fold, respectively.

Based on these results, the MOA responsible for increased liver weight in rats following exposure to X11719474 was sulfoxaflor- and phenobarbital-like, as evidenced by the CAR- and PXR-related molecular, enzymatic and proliferative responses. These data support the conclusion that X11719474 is an agonist ligand for CAR and PXR, and this activation results in the observed phenobarbital-like responses in the liver (Geter et al., 2010b).

Studies with X11721061 (plant and animal metabolite of sulfoxaflor)

In an acute oral toxicity study conducted according to OECD test guideline 425 ("up and down procedure"), female Fischer 344 rats received X11721061 (purity 99%, clear, colourless liquid) at a single dose of 100, 2000 or 5000 mg/kg bw by gavage. Single animals were treated sequentially.

Depending on the survival or death of the treated animal, the dose level administered to the next animal was increased or decreased, respectively. The rats were monitored daily for mortality and clinical signs for a 2-week period, and body weights were recorded on days 1, 7 and 14. On day 15, surviving animals were killed, necropsied and examined for gross pathological changes.

One of the females dosed with 5000 mg/kg bw died; additional mortalities were observed at a dose level of 2000 mg/kg bw (two of three females) within 1 day after the dose administration. Two of two females dosed with 1000 mg/kg bw survived. All animals that died showed clinical signs of toxicity and red intestines upon necropsy. Surviving animals showed clinical signs (e.g. piloerection, hypoactivity, hunched posture), but recovered by day 2, at the latest. No abnormalities were noted in surviving animals. The estimated acute oral LD_{50} in female rats was 2000 mg/kg bw (Durando, 2010b).

In a reverse gene mutation assay in bacteria conducted according to OECD test guideline 471 (1997), *Salmonella typhimurium* (strains TA98, TA100, TA1535, TA1537) and *Escherichia coli* (strain WP2*uvr*A) were exposed to X11721061 (purity 99%), using DMSO as solvent, in the presence and absence of S9 metabolic activation in two independent sets of experiments. Doses were selected based on the guideline limit dose and results of an initial experiment. For the initial preincubation test using doses of up to and including 5000 µg/plate, two plates were used for each strain, condition and dose. Vehicle and positive controls were included in each experiment. The independent repeat test utilized basically the same conditions as the initial test, but the dose levels were adjusted, and three plates were used for each strain, condition and dose. Doses up to and including 5000 µg/plate did not cause any bacteriotoxic effects. Total bacteria counts remained unchanged, and no inhibition of growth was observed.

No evidence for mutagenic activity of X11721061 was seen. No biologically relevant increase in the mutant count, in comparison with the negative controls, was observed. The positive controls induced the appropriate responses in the corresponding strains. Therefore, X11721061 was considered to be not mutagenic in the bacterial strains tested, in either the presence or absence of metabolic activation (Dakoulas & VanDyke, 2009).

In an in vitro mammalian cell gene mutation test conducted according to OECD test guideline 476 (1997), X11721061 (purity 99%) dissolved in distilled water was tested for its ability to induce forward mutations at the *HPRT* locus in CHO cells. Two independent sets of experiments were conducted in the presence and absence of S9 metabolic activation. Based on the guideline limit dose (10 mmol/l), concentrations of 120–1920 µg/ml were used in the main study both with and without metabolic activation, and the same concentrations were used for the independent repeats. EMS and 20-MCA served as positive controls in the experiments without and with metabolic activation, respectively. The cells were treated for 4 hours in both experiments, without and with metabolic activation. After this, the incubation media were replaced by culture medium, and the cells were incubated (and subcultured every 2–3 days) for about 8 days for expression of mutant cells. This was followed by incubation of cells for 7–9 days in selection medium containing 6-thioguanine.

No increase in mutant frequency was observed in either the initial or confirmatory study. In contrast to this, the positive control substances EMS and 20-MCA resulted in a marked increase in mutant frequency. Based on the results of the study, X11721061 was considered to be not mutagenic in the CHO/HPRT forward mutation assay, in either the presence or absence of metabolic activation (Schisler, 2010a).

In an in vitro mammalian chromosomal aberration test conducted according to OECD test guideline 473 (1997), the clastogenic potential of X11721061 (purity 99%) dissolved in distilled water

was tested in rat lymphocytes (whole blood). Based on the results of the mitotic index, concentrations of 480, 960 and 1920 μg/ml were used with and without metabolic activation in an experiment with 4 hours of treatment and harvest at 24 hours (i.e. 20 hours post-exposure). In addition, with 24 hours of treatment without S9 mix, concentrations of 120, 240 and 480 μg/ml were chosen. Vehicle and positive controls (cyclophosphamide and mitomycin C for the test with and without metabolic activation, respectively) were included to demonstrate the sensitivity of the test system. Cultures were set up in duplicate. After slide preparation and staining of the cells, 200 metaphases per dose and treatment condition were analysed for chromosomal aberrations.

None of the cultures treated with X11721061 in the absence or in the presence of S9 mix showed any biologically relevant or statistically significant increase in the numbers of aberrant metaphases. No change in the frequency of polyploid metaphases was observed. The positive controls mitomycin C and cyclophosphamide induced clastogenic effects and demonstrated the sensitivity of the test system and the activity of the S9 mix used. Based on the results of this test, X11721061 was considered not to be clastogenic for mammalian cells in vitro, in either the presence or absence of metabolic activation (Schisler & Geter, 2010).

In a non-guideline, non-GLP palatability study, groups of three male F344/DuCrl rats were fed diets containing X11721061 (purity 98%) at a concentration of 2500, 5000 or 10 000 ppm (equal to approximately 150–175, 323–372 and 547–772 mg/kg bw per day) for up to 7 days to evaluate palatability. Parameters evaluated were daily cage-side observations, body weights, feed consumption and gross examinations. Liver weight was recorded.

All animals survived the treatment period without any clinical signs of toxicity. Body weight development and feed intake were normal. No gross findings were observed. Liver weights were increased dose relatedly (as a result of the lack of a control group, the extent could not be determined).

Therefore, under the conditions of this study, X11721061 was sufficiently palatable at the concentrations tested for studies of repeated administration (Heward, 2010c).

In a study of toxicity conducted according to OECD test guideline 407, groups of five male and five female F344/DuCrl rats were fed diets containing X117721061 (purity 99%) at a concentration of 0, 1000, 3000 or 8000 ppm (equal to 0, 79, 236 and 622 mg/kg bw per day for males and 0, 82, 244 and 649 mg/kg bw per day for females) for 28 days. Animals were observed daily for mortality and clinical signs. A detailed physical examination was performed weekly. Body weight and feed consumption were recorded frequently throughout the study. Ophthalmoscopic examinations were performed pretest and prior to terminal necropsy. Blood samples for haematology and clinical chemistry determinations were collected before final necropsy, whereas urine samples were collected during the last week of the study. At study termination, all animals were necropsied, selected organs were weighed and a range of tissues were taken, fixed and examined microscopically.

No mortalities were observed throughout the treatment period. However, a few animals died during the terminal blood collection. No treatment-related clinical signs of toxicity or other clinical abnormalities were reported.

Body weight loss in the top-dose females and low feed intake in the top-dose males and females (approximately 12–13%) were observed during the first 3 days of treatment. Feed intake and body weight development were normal during later periods in the top-dose animals and in all other dose groups during the whole study period.

No treatment-related effect was noted during ophthalmoscopic examination. Haematological and clinical chemistry analyses and urinalysis showed no consistent changes attributable to treatment.

Table 42. Summary of selected findings in rats in a 28-day study with metabolite X117721061

	Males				Females			
	Dietary concentration (ppm)							
	0	1000	3000	8000	0	1000	3000	8000
Dose (mg/kg bw per day), day 27	0	79	236	622	0	82	244	649
Body weight, terminal (g)	211	212	205	203	131	132	129	128
Liver weight, absolute (g)	6.74	6.68	6.74	7.17	3.88	4.12	3.93	4.09
Liver weight, relative (% of body weight)	3.18	3.16	3.29	3.52	2.97	3.12	3.06	3.19*

From Heward (2010d)
* $P < 0.05$

Macroscopic examination did not reveal treatment-related changes. Terminal body weights of top-dose males were slightly lower than in control animals. Absolute liver weight of top-dose males and females and relative liver weight of top-dose males were slightly, although not statistically significantly, higher, and the relative liver weight of top-dose females was significantly increased (Table 42). No treatment-related histopathological changes were reported.

The NOAEL was 3000 ppm (equal to 236 mg/kg bw per day), based on reduced feed intake in both sexes and a subsequent slight body weight loss in females at 8000 ppm (equal to 622 mg/kg bw per day) during the first 3 days of treatment (Heward, 2010d).

Studies with X11596066 (animal metabolite of sulfoxaflor in hens and goats)

In an acute oral toxicity study conducted according to OECD test guideline 425 ("up and down procedure"), female Fischer 344 rats received X11596066 (purity 98%) in 0.5% aqueous methylcellulose at a single dose of 2000 mg/kg bw by gavage. Single animals were treated sequentially. Depending on the survival or death of the treated animal, the dose level administered to the next animal was increased or decreased, respectively. The rats were monitored daily for mortality and clinical signs for a 2-week period, and body weights were recorded on days 1, 7 and 14. On day 15, surviving animals were killed, necropsied and examined for gross pathological changes.

All five females dosed with 2000 mg/kg bw survived and gained weight during the study. Clinical signs (piloerection) were noted in two animals on the day of dosing. No gross abnormalities were noted during necropsy. The estimated acute oral LD_{50} in rats was greater than 2000 mg/kg bw in females (Durando, 2010c).

In a reverse gene mutation assay in bacteria conducted according to OECD test guideline 471 (1997), *Salmonella typhimurium* (strains TA98, TA100, TA1535, TA1537) and *Escherichia coli* (strain WP2*uvr*A) were exposed to X11596066 (purity 98%), using DMSO as solvent, in the presence and absence of S9 metabolic activation, in two independent sets of experiments. Doses were selected based on the guideline limit dose and results of an initial experiment. For the initial preincubation test using doses of up to and including 5000 µg/plate, two plates were used for each strain, condition and dose. Vehicle and positive controls were included in each experiment. The independent repeat test utilized basically the same conditions as the initial test, but the dose levels were adjusted, and three plates were used for each strain, condition and dose. Doses up to and including 5000 µg/plate did not cause any bacteriotoxic effects. Total bacteria counts remained unchanged, and no inhibition of growth was observed.

No evidence for mutagenic activity of X11596066 was seen. No biologically relevant increase in the mutant count, in comparison with the negative controls, was observed. The positive controls induced the appropriate responses in the corresponding strains. Therefore, X11596066 was considered to be not mutagenic in the bacterial strains tested, in either the presence or absence of metabolic activation (Dakoulas & VanDyke, 2010a).

Studies with X11579457 (soil metabolite of sulfoxaflor)

In an acute oral toxicity study conducted according to OECD test guideline 425 ("up and down procedure"), female Fischer 344 rats received X11579457 (purity 99%) in 0.5% aqueous methylcellulose at a single dose of 2000 mg/kg bw by gavage. Single animals were treated sequentially. Depending on the survival or death of the treated animal, the dose level administered to the next animal was increased or decreased, respectively. The rats were monitored daily for mortality and clinical signs for a 2-week period, and body weights were recorded on days 1, 7 and 14. On day 14, surviving animals were killed, necropsied and examined for gross pathological changes.

All five females dosed with 2000 mg/kg bw survived. Clinical signs were noted in one animal on the day of compound administration. No gross abnormalities were observed when animals were necropsied. The acute oral LD_{50} in rats was greater than 2000 mg/kg bw in females (Durando, 2010d).

In a reverse gene mutation assay in bacteria conducted according to OECD test guideline 471 (1997), *Salmonella typhimurium* (strains TA98, TA100, TA1535, TA1537) and *Escherichia coli* (strain WP2*uvr*A) were exposed to X11579457 (purity 99%), using distilled water as solvent, in the presence and absence of S9 metabolic activation in two independent sets of experiments. Doses were selected based on the guideline limit dose and results of an initial experiment. For the initial preincubation test using doses of up to and including 5000 µg/plate, two plates were used for each strain, condition and dose. Vehicle and positive controls were included in each experiment. The independent repeat test utilized basically the same conditions as the initial test, but the dose levels were adjusted, and three plates were used for each strain, condition and dose. Doses up to and including 5000 µg/plate did not cause any bacteriotoxic effects. Total bacteria counts remained unchanged, and no inhibition of growth was observed.

No evidence for mutagenic activity of X11579457 was seen. No biologically relevant increase in the mutant count, in comparison with the negative controls, was observed. The positive controls induced the appropriate responses in the corresponding strains. Therefore, X11579457 was considered to be not mutagenic in the bacterial strains tested, in either the presence or absence of metabolic activation (Dakoulas & VanDyke, 2010b).

In an in vitro mammalian cell gene mutation test conducted according to OECD test guideline 476 (1997), X11579457 (purity 99%) dissolved in distilled water was tested for its ability to induce forward mutations at the *HPRT* locus in CHO cells. Two independent sets of experiments were conducted in the presence and absence of S9 metabolic activation. Based on the guideline limit dose (10 mmol/l), concentrations of 157.8–2525 µg/ml were used in the main study both with and without metabolic activation, and the same concentrations were used for the independent repeats. EMS and 20-MCA served as positive controls in the experiments without and with metabolic activation, respectively. The cells were treated for 4 hours in both experiments, without and with metabolic activation. After this, the incubation media were replaced by culture medium, and the cells were incubated (and subcultured every 2–3 days) for about 8 days for expression of mutant cells. This was followed by incubation of cells for 7–9 days in selection medium containing 6-thioguanine.

No increase in mutant frequency was observed in either the initial or confirmatory study. In contrast to this, the positive control substances EMS and 20-MCA resulted in a marked increase in mutant frequency. Based on the results of the study, X11579457 was considered to be not mutagenic in the CHO/HPRT forward mutation assay, in either the presence or absence of metabolic activation (Schisler, 2010c).

In an in vitro mammalian chromosomal aberration test conducted according to OECD test guideline 473 (1997), the clastogenic potential of X11579457 (purity 99%) dissolved in distilled water was tested in rat lymphocytes (whole blood). Based on the results of the mitotic index, concentrations

of 631.3, 1262.5 and 2525 µg/ml were used with and without metabolic activation in an experiment with 4 hours of treatment and harvest at 24 hours and in an experiment with 24 hours of treatment without S9 mix. Vehicle and positive controls (cyclophosphamide and mitomycin C for the test with and without metabolic activation, respectively) were included to demonstrate the sensitivity of the test system. Cultures were set up in duplicate. After slide preparation and staining of the cells, 200 metaphases per dose and treatment condition were analysed for chromosomal aberrations.

None of the cultures treated with X11579457 in the absence or in the presence of S9 mix showed any biologically relevant or statistically significant increase in the numbers of aberrant metaphases. No change in the frequency of polyploid metaphases was observed. The positive controls mitomycin C and cyclophosphamide induced clastogenic effects and demonstrated the sensitivity of the test system and the activity of the S9 mix used. Based on the results of this test, X11579457 was considered not to be clastogenic for mammalian cells in vitro, in either the presence or absence of metabolic activation (Schisler, 2010b).

Studies with X11519540 (soil and animal metabolite of sulfoxaflor)

In an acute oral toxicity study conducted according to OECD test guideline 425 ("up and down procedure"), female Fischer 344 rats received X11519540 (purity 98%) in 0.5% aqueous methylcellulose at a single dose of 320, 1000 or 2000 mg/kg bw by gavage. Single animals were treated sequentially. Depending on the survival or death of the animal, the dose level administered to the next animal was increased or decreased, respectively. The rats were monitored daily for mortality and clinical signs for a 2-week period, and body weights were recorded on days 1, 7 and 14. On day 14, surviving animals were killed, necropsied and examined for gross pathological changes.

One animal dosed with 2000 mg/kg bw died within 1 day of test substance administration; additional mortalities were observed at a dose level of 1000 mg/kg bw (three of three females, within 2 days). Clinical signs of toxicity (such as hyperactivity/hypoactivity, abnormal posture or gait, salivation) were observed. At necropsy, red discoloration of intestines was observed in the decedents. All animals ($n = 3$) dosed with 320 mg/kg bw survived, and body weight gain was normal. The estimated acute oral LD_{50} in rats was 565.7 mg/kg bw in females (Durando, 2010e).

In a reverse gene mutation assay in bacteria conducted according to OECD test guideline 471 (1997), *Salmonella typhimurium* (strains TA98, TA100, TA1535, TA1537) and *Escherichia coli* (strain WP2*uvr*A) were exposed to X11519540 (purity 98%), using DMSO as solvent, in the presence and absence of S9 metabolic activation in two independent sets of experiments. Doses were selected based on the guideline limit dose and results of an initial experiment. For the initial preincubation test using doses of up to and including 5000 µg/plate, two (initial experiment) or three plates (second experiment) were used for each strain, condition and dose. Vehicle and positive controls were included in each experiment. The independent repeat test utilized basically the same conditions as the initial test, but the dose levels were adjusted. Doses up to and including 5000 µg/plate did not cause any bacteriotoxic effects. Total bacteria counts remained unchanged, and no inhibition of growth was observed.

No evidence for mutagenic activity of X11519540 was seen. No biologically relevant increase in the mutant count, in comparison with the negative controls, was observed. The positive controls induced the appropriate responses in the corresponding strains. Therefore, X11519540 was considered to be not mutagenic in the bacterial strains tested, in either the presence or absence of metabolic activation (Dakoulas & VanDyke, 2010c).

In an in vitro mammalian cell gene mutation test conducted according to OECD test guideline 476 (1997), X11519540 (98%) dissolved in DMSO was tested for its ability to induce forward mutations at the *HPRT* locus in CHO cells. Two independent sets of experiments were conducted in the presence and absence of S9 metabolic activation. Based on the guideline limit dose (10 mmol/l),

concentrations of 158.8–2540 µg/ml were used in the main study both with and without metabolic activation, and the same concentrations were used for the independent repeats. EMS and 20-MCA served as positive controls in the experiments without and with metabolic activation, respectively. The cells were treated for 4 hours in both experiments, without and with metabolic activation. After this, the incubation media were replaced by culture medium, and the cells were incubated (and sub-cultured every 2–3 days) for about 8 days for expression of mutant cells. This was followed by incubation of cells for 7–9 days in selection medium containing 6-thioguanine.

No increase in mutant frequency was observed in either the initial or confirmatory studies. In contrast to this, the positive control substances EMS and 20-MCA resulted in a marked increase in mutant frequency. Based on the results of the study, X11519540 was considered to be not mutagenic in the CHO/HPRT forward mutation assay, in either the presence or absence of metabolic activation (Schisler, 2010e).

In an in vitro mammalian chromosomal aberration test conducted according to OECD test guideline 473 (1997), the clastogenic potential of X11519540 (purity 98%) dissolved in DMSO was tested in rat lymphocytes (whole blood). Based on the results of the mitotic index, concentrations of 635, 1270 and 2540 µg/ml were used with and without metabolic activation in an experiment with 4 hours of treatment and harvest at 24 hours (i.e. 20 hours post-exposure). In addition, with 24 hours of treatment without S9 mix, concentrations of 158.8, 317.5 and 635 µg/ml were chosen. Vehicle (DMSO) and positive controls (cyclophosphamide and mitomycin C for the test with and without metabolic activation, respectively) were included to demonstrate the sensitivity of the test system. Cultures were set up in duplicate. After slide preparation and staining of the cells, 200 metaphases per dose and treatment condition were analysed for chromosomal aberrations.

None of the cultures treated with X11519540 in the absence or in the presence of S9 mix showed any biologically relevant or statistically significant increase in the numbers of aberrant metaphases. No change in the frequency of polyploid metaphases was observed. The positive controls mitomycin C and cyclophosphamide induced clastogenic effects and demonstrated the sensitivity of the test system and the activity of the S9 mix used. Based on the results of this test, X11519540 was considered not to be clastogenic for mammalian cells in vitro, in either the presence or absence of metabolic activation (Schisler, 2010d).

In a non-guideline, non-GLP palatability study, groups of three male F344/DuCrl rats were fed diets containing X11519540 (purity 98%) at a concentration of 2500, 5000 or 10 000 ppm (equal to 200, 355 and 319 mg/kg bw per day) for up to 7 days to evaluate palatability. Parameters evaluated were daily cage-side observations, body weights, feed consumption and gross examinations. Liver samples of low-dose animals were processed for histopathological evaluation and examined microscopically.

Feed consumption was reduced by 29.6%, 46.4% or 72.3% in animals given 2500, 5000 or 10 000 ppm, respectively, relative to pre-administration days −4 to −1. This was attributed to decreased palatability of the test compound in rodent feed. All animals fed 10 000 ppm had marked body weight loss and low feed intake by day 4 and were humanely euthanized on that day. Body weight losses were observed in the low- and intermediate-dose groups by day 4, which recovered slightly by day 8. Liver weight was increased in low and intermediate dose group animals (by approximately 60–70% compared with historical control values). In liver sections of low-dose animals, the following observations were noted: centrilobular to midzonal hepatocellular hypertrophy with altered tinctorial properties, very slight increase in mitotic figures and very slight multifocal individual cell necrosis.

Therefore, under the conditions of this study, X11519540 was not sufficiently palatable at the concentrations tested for studies of repeated adminstration. Dose levels to be used in future dietary toxicity studies should be below 250 mg/kg bw per day (Sura & Brooks, 2010).

In a toxicity study conducted according to OECD test guideline 407 (2008), groups of five male and five female F344/DuCrl rats were given diets containing X11519540 (purity 98%) at a concentration of 0, 100, 300, 1000 or 2000 ppm (equal to 0, 7.7, 23.1, 74 and 140 mg/kg bw per day in males and 0, 8.5, 24.9, 77.2 and 152 mg/kg bw per day in females) for 28 days. Animals were observed daily for mortality and clinical signs. Physical examinations were performed weekly. Body weight and feed consumption were recorded once weekly. During the acclimatization phase, all animals were subjected to an ophthalmic examination; all animals were re-examined prior to scheduled necropsy. Haematology, clinical chemistry and urine parameters were determined at the end of the study. All animals were necropsied, selected organs were weighed and a range of tissues were taken, fixed and examined microscopically. A liver piece from each animal was collected for gene expression analysis.

No dose-related effect on mortality rate was reported. There were no treatment-related effects on clinical signs, ophthalmic observations or coagulation parameters. Males and females given 1000 or 2000 ppm exhibited reduced body weight gain and reduced feed intake compared with controls.

In animals treated with 1000 or 2000 ppm, parameters for red blood cells/haemoglobin were decreased. In animals treated with 300 ppm, these parameters were decreased too; however, they stayed within the range of historical control data, and hence these changes were considered to be non-adverse. In animals treated with 300 ppm, total protein, albumin and cholesterol levels were increased. In animals dosed with 1000 or 2000 ppm, additionally, the following clinical chemistry parameters were increased: γ-glutamyl transpeptidase, ALT and AST and, additionally in females, globulin level. Urinary pH was decreased in males dosed with 300 ppm and in both sexes at 100 and 2000 ppm. Males treated with 2000 ppm had slightly higher urinary protein levels than controls.

Males and females in all treatment groups had increased (absolute and relative) adrenal and liver weights. Terminal body weight was lower in animals of the 1000 and 2000 ppm dose groups compared with controls. Liver size was increased in animals treated with 300 ppm and above.

Animals in all treatment groups showed centrilobular/midzonal hypertrophy, which increased in severity (very slight to moderate). Males in all treatment groups had an increased number of mitotic figures in liver sections; females showed this finding at 300 ppm and above. Multifocal necrosis of individual hepatocytes was noted in both sexes at 300 ppm and above. At 1000 ppm and above, multifocal hepatocellular necrosis with accompanying inflammation was noted. An increased incidence of multifocal degeneration of tubules in the kidneys in males (300, 1000, 2000 ppm) and females (2000 ppm) was noted. Males from all dose levels and females given 1000 or 2000 ppm had diffuse follicular cell hypertrophy of the thyroid gland. Males and females given 300, 1000 or 2000 ppm had treatment-related very slight or slight hypertrophy of the zona fasciculata of the adrenal glands. In addition, males from all dose levels and females given 300, 1000 or 2000 ppm had treatment-related very slight or slight increased vacuolation of the adrenal cortex (zona glomerulosa, zona fasciculata and zona reticularis). Males given 1000 or 2000 ppm and females given 2000 ppm had very slight diffuse acinar cell hypertrophy of the submandibular salivary gland.

Males and females given 1000 or 2000 ppm had treatment-related very slight or slight erythroid cell hyperplasia of the bone marrow. Males given 2000 ppm had a treatment-related increase in the incidence of very slight extramedullary haematopoiesis (erythroid cell) of the spleen. The bone marrow and spleen effects were interpreted to be reflective of a regenerative response to the lower red blood cell counts, haemoglobin concentrations and haematocrits in males and females given 1000 or 2000 ppm.

Males and females given 1000 or 2000 ppm had treatment-related very slight or slight atrophy of the mesenteric adipose tissue. Females given 1000 or 2000 ppm had treatment-related very slight or slight decreased size of the uterus. Females given 2000 ppm also had treatment-related slightly decreased size of the cervix and vagina. The atrophy of mesenteric adipose tissue and decreased size

of the female reproductive tract were interpreted to be secondary to lower feed consumption and lower body weight gains of animals given 1000 or 2000 ppm.

Toxicokinetic analysis indicated that systemic exposure to the test compound deviated from linearity (plasma AUC versus mean achieved daily dose level) between the 100 and 300 ppm groups. The plasma elimination half-life was between 24 and 35 hours in the 1000 and 3000 ppm group animals. It could not be determined in the 100 and 300 ppm dose groups. Urinary excretion within 24 hours was approximately 47%/51%, 31%/28%, 18%/15% or 13%/11% (males/females) of the calculated mean daily dose in the 100, 300, 1000 or 2000 ppm dose groups.

Targeted gene expression analysis of *Cyp2b1*, *Cyp2b2* and *Cyp3a3* in the liver tissue showed significantly elevated transcripts at 100, 300 and 1000 ppm (2000 ppm was not tested for these end-points). These data suggest that X11519540 may stimulate gene expression consistent with CAR activation.

The NOAEL was less than 100 ppm (equal to 7.7 mg/kg bw per day) in males, based on effects in liver (increased serum cholesterol), thyroid (follicular cell hypertrophy) and adrenals (increased vacuolation of the cortex) (Stebbins et al., 2010b).

3. Observations in humans

There were no reports of adverse health effects in manufacturing plant personnel. Also, there were no reports of poisonings with sulfoxaflor.

Comments

Biochemical aspects

In rats given sulfoxaflor labelled with ^{14}C at the pyridine ring orally by gavage, absorption was rapid and accounted for at least 93% of the total recovered radioactivity after a single dose of 5 mg/kg bw or 100 mg/kg bw; the maximum plasma concentrations of radiolabelled material were reached after 0.5–1.6 hours and after 1.3–2.3 hours, respectively. Radiolabel was widely distributed throughout the body. Elimination of the radiolabel was mainly via the urine (≥ 92%). After intravenous administration, faecal excretion accounted for up to 9% of total excretion. Elimination of the radiolabel from plasma was bi-exponential, with most of the elimination occurring during the α phase, with a half-life of 4–6 hours, whereas the half-life of the β phase was 39–45 hours. Residues in tissues 168 hours after a single oral or intravenous dose as well as after repeated oral dosing accounted for less than 1.3% of the administered dose, with liver, kidney and erythrocytes containing the highest concentrations of residues.

Sulfoxaflor was metabolized to only a very limited extent. The metabolism included oxidative cleavage of the parent molecule, leading to metabolite X11721061, which was subsequently conjugated with glucuronic acid. This was the only metabolite identified in urine, accounting for 3–4% of the administered dose.

Toxicological data

The LD_{50} in rats treated orally with sulfoxaflor was 1000 mg/kg bw. The dermal LD_{50} in rats was greater than 5000 mg/kg bw, and the inhalation LC_{50} in rats was greater than 2.09 mg/l. Sulfoxaflor was not a skin irritant in rabbits, was not irritating to the eye of rabbits and was not a skin sensitizer in the local lymph node assay in mice.

At least in part as a result of its unpleasant smell, sulfoxaflor is of limited oral palatability, so that repeated-dose studies by dietary as well as gavage administration were dose limited by effects on feed intake and consequent body weight reductions.

Following repeated administration of sulfoxaflor to mice and rats, the liver was the main target organ, and males were affected more than females. The effects noted at lower doses (increased liver weights, hepatocellular hypertrophy) were consistent with the induction of hepatic cytochrome P450, whereas effects observed at higher doses included hepatocellular degeneration or necrosis and related clinical chemistry findings (e.g. increased serum levels of liver enzymes, cholesterol or triglycerides). In mice, the adrenals were an additional target, with hypertrophy and/or vacuolization of the zona fasciculata. In dogs, gavage administration gave the highest achievable doses, but the only effects were decreases in feed consumption and body weight gain and increased incidences of soft or watery faeces.

In a 28-day study in mice, the NOAEL was 300 ppm (equal to 43.9 mg/kg bw per day), based on effects in the liver (increased serum ALT, vacuolation/fatty change of hepatocytes) at 1500 ppm (equal to 230 mg/kg bw per day). In a 90-day study in mice, the NOAEL was 100 ppm (equal to 12.8 mg/kg bw per day), based on effects in the liver (vacuolation/fatty change of hepatocytes) and the adrenals (hypertrophy and/or vacuolation of the zona fasciculata) observed in males at 750 ppm (equal to 98 mg/kg bw per day) and in females at 1500 ppm (equal to 247 mg/kg bw per day).

In a 28-day study in rats, the NOAEL was 300 ppm (equal to 24.8 mg/kg bw per day), based on marginal liver toxicity (increased serum cholesterol and total protein levels) in males at 1000 ppm (equal to 79.4 mg/kg bw per day). In a 90-day study in rats, the NOAEL was 100 ppm (equal to 6.36 mg/kg bw per day), based on effects in the liver (increased serum cholesterol level, vacuolation/fatty change of hepatocytes) in males at 750 ppm (equal to 47.6 mg/kg bw per day). After a 28-day recovery phase, very slight histopathological changes in the liver (hypertrophy and fatty change of hepatocytes) were seen in males at 1500 ppm (equal to 94.9 mg/kg bw per day).

In a 90-day oral gavage study in dogs, the NOAEL was 6 mg/kg bw per day, based on decreased feed consumption and decreased body weights during the 1st week of exposure at 10 mg/kg bw per day. After reduction of this dose to 6 mg/kg bw per day on study day 5, no treatment-related adverse effects were observed. In a 1-year oral gavage study in dogs, the NOAEL was 6 mg/kg bw per day, the highest dose tested. The increased incidences of soft/watery faeces in two males at this dose were not considered adverse, as these changes were not accompanied by any other toxicological effect. Also, the slight decreases in feed consumption and body weight in two females during the first 2 weeks of dosing at 6 mg/kg bw per day were not considered adverse, as there were no changes during the remainder of the study. The overall NOAEL for the 90-day and 1-year studies was 6 mg/kg bw per day.

Long-term studies of toxicity and carcinogenicity were conducted in mice and rats. In an 18-month study of carcinogenicity in mice, the NOAEL for carcinogenicity was 100 ppm (equal to 10.4 mg/kg bw per day), based on an increased incidence of hepatocellular adenomas and/or carcinomas in males at 750 ppm (equal to 79.6 mg/kg bw per day). The NOAEL for non-neoplastic changes was 100 ppm (equal to 10.4 mg/kg bw per day), based on liver toxicity (vacuolation/fatty change of hepatocytes) in males at 750 ppm (equal to 79.6 mg/kg bw per day).

In a series of mechanistic studies in mice, including C57BL/6J knockout mice for PXR and CAR and C57BL/6J mice "humanized" for PXR and CAR, it was demonstrated that sulfoxaflor was a relatively potent phenobarbital-like inducer of hepatic P450 enzymes via activation of CAR and possibly, to some extent, PXR. This was apparent at the mRNA, protein and enzyme activity level. Activation of the mouse CAR (and possibly PXR) resulted in increased hepatocyte hypertrophy and proliferation. The human CAR (and possibly PXR) supported modest P450 induction and hepatic hypertrophy by sulfoxaflor, but did not support any effect on hepatocyte proliferation.

In a 24-month study of toxicity and carcinogenicity in rats, the NOAEL for carcinogenicity was 100 ppm (equal to 4.24 mg/kg bw per day), based on an increased incidence of hepatocellular

adenomas in males at 500 ppm (equal to 21.3 mg/kg bw per day). Also at 500 ppm, there was an increased incidence of bilateral Leydig (interstitial) cell adenomas of the testes, whereas there was no effect on the incidence of combined unilateral/bilateral Leydig cell adenomas. The size and weight of the testes and the size of Leydig cell adenomas were increased at 100 and 500 ppm and were associated with the secondary changes in the testes and epididymides listed below. The NOAEL for non-neoplastic effects was 25 ppm (equal to 1.04 mg/kg bw per day), based on changes in the testes (increased testes weights, increased incidence of severe bilateral atrophy of seminiferous tubules) and epididymides (decreased epididymal weights, increased incidence of severe bilateral decreased spermatic elements of the epididymides) in males at 100 ppm (equal to 4.24 mg/kg bw per day). In females, the NOAEL for non-neoplastic effects was 100 ppm (equal to 5.13 mg/kg bw per day), based on hepatocellular degeneration at 750 ppm (equal to 39.0 mg/kg bw per day).

In a mechanistic study on liver tumorigenesis in rats, 3-day or 7-day exposure to sulfoxaflor at dietary concentrations up to 1500 ppm (equal to 83–102 mg/kg bw per day) resulted in increased liver weights, increased cell proliferation in the centrilobular and midzonal regions of the hepatic lobules, marked induction of *Cyp2b1* gene expression and hepatic activities of PROD and BROD, and moderate induction of *Cyp2b2* and *Cyp3a3* expression levels. The pattern of changes was phenobarbital-like, as evidenced by the CAR- and PXR-related molecular, enzymatic and proliferative responses.

The Meeting concluded that for the liver tumours in both mice and rats, there was sufficient evidence to support the proposed phenobarbital-like MOA. In particular, sulfoxaflor exhibited clearly higher activity towards rodent CAR than towards human CAR. The marked qualitative and quantitative species differences in the key events in the MOA for neoplasia in response to CAR activation allowed for the conclusion that the sulfoxaflor-induced liver tumours in rats and mice are not relevant to humans (see Appendix 1).

In a mechanistic study conducted to examine the potential MOA for the Leydig cell effects seen in the rat carcinogenicity study, 8-week exposure of male Fischer 344 rats to sulfoxaflor at dietary concentrations up to 500 ppm (equal to 28 mg/kg bw per day) resulted in decreased serum prolactin and increased serum LH and testosterone levels and in decreased testis LHR and prolactin receptor gene expression at week 4, but not at week 2 or week 8. Treatment had no effect on the percentage of Leydig cells with intracellular staining of LHR, biliary excretion of [^{14}C]testosterone, serum 17β-estradiol level or any measured gene in the steroidogenic pathway. Because Fischer 344 rats are particularly susceptible to effects on Leydig cells, analogous treatment of male Sprague-Dawley rats was performed, resulting in increased serum LH and testosterone levels at week 2 and a decrease in serum prolactin level at week 4.

In a mechanistic study using intracerebral microdialysis in rats, sulfoxaflor infusion (0.4 and 2 mmol/l) evoked dose-related increases in the extracellular level of dopamine in the mediobasal hypothalamus, with a maximal rise of 39%, 40 minutes after the onset of infusion at 2 mmol/l.

In a further mechanistic study on Leydig cell effects, sulfoxaflor did not bind to the ERα and had weak binding affinity to the AR, whereas it did not show any agonism or antagonism in the ER and AR transactivation assays. In addition, there was no evidence for aromatase inhibition by sulfoxaflor.

Although the proposed MOA—that sulfoxaflor can act as a dopamine agonist in the central nervous system and may inhibit prolactin release in the pituitary (an MOA for the induction of Leydig cell tumours that is considered to be not relevant to humans)—has not been completely demonstrated, the Meeting concluded that the increased incidences of bilateral Leydig cell adenomas in male rats are of low relevance to humans, as there are large qualitative and quantitative differences between rats and humans regarding Leydig cell responses to hormonal stimuli (see Appendix 1). In addition, these effects occurred only at high doses, did not occur in mice and would be anticipated to exhibit a threshold. As a consequence, the secondary changes in the testes and epididymides would not be relevant to the dietary risk assessment of sulfoxaflor.

Sulfoxaflor was tested for genotoxicity in vitro and in vivo in an adequate range of assays. It was not found to be genotoxic in mammalian or microbial test systems.

The Meeting concluded that sulfoxaflor was unlikely to be genotoxic.

On the basis of the absence of genotoxicity, the human non-relevance of the liver tumours in both mice and rats and the fact that the Leydig cell responses observed in rats are unlikely to be relevant to humans (see Appendix 1), the Meeting concluded that sulfoxaflor is unlikely to pose a carcinogenic risk to humans at dietary exposure levels.

In a reproduction/developmental toxicity screening study in rats, the NOAEL for both parental toxicity and effects on offspring was 100 ppm (equal to 8.26 mg/kg bw per day), based on decreased body weight gains in females during the 1st week of gestation and reduced pup survival at 500 ppm (equal to 40.7 mg/kg bw per day).

In a two-generation reproductive toxicity study in rats, the NOAEL for effects on fertility was 400 ppm (equal to 24.6 mg/kg bw per day), the highest dose tested. The NOAEL for parental toxicity was 100 ppm (equal to 6.07 mg/kg bw per day), based on liver toxicity (increase in vacuolation/fatty change of centrilobular hepatocytes) in F_0 males at 400 ppm (equal to 24.6 mg/kg bw per day). The NOAEL for offspring toxicity was 100 ppm (equal to 6.07 mg/kg bw per day), based on reduced pup survival and delayed preputial separation (puberty onset) in F_2 males at 400 ppm (equal to 24.6 mg/kg bw per day).

In a cross-fostering study conducted to assess whether the observed effects of sulfoxaflor on neonatal survival in rats resulted from in utero and/or lactational exposure, all offspring from dams exposed to sulfoxaflor (1000 ppm, equal to 60–81 mg/kg bw per day) prior to birth died by PND 4, irrespective of whether they were cross-fostered to control or treated foster dams. There was no effect on survival for pups exposed only after birth. Thus, the effect of sulfoxaflor on pup survival was due to in utero, not lactational, exposure.

In a developmental toxicity study in rats, the NOAEL for maternal toxicity was 150 ppm (equal to 11.5 mg/kg bw per day), based on decreased body weight and body weight gain and decreased feed consumption at 1000 ppm (equal to 70.2 mg/kg bw per day). The NOAEL for developmental toxicity was 150 ppm (equal to 11.5 mg/kg bw per day), based on increases in several fetal abnormalities (forelimb flexure, hindlimb rotation, bent clavicle, fused sternebrae, convoluted ureter and hydroureter) at 1000 ppm (equal to 70.2 mg/kg bw per day).

A series of special studies conducted to determine the critical window of developmental susceptibility of rat fetuses demonstrated that late gestational exposure (i.e. from GD 20 to GD 21 or 22) of dams to sulfoxaflor (1000 ppm, equal to 36–39 mg/kg bw per day) resulted in reduced neonatal survival and limb abnormalities seen in pups at PNDs 1–3, whereas no limb abnormalities were observed at PND 4 in the same litters. Offspring from dams exposed (at 1000 ppm, equal to 43–77 mg/kg bw per day) up to GD 19 did not show any limb abnormalities or reduced neonatal survival.

Histopathological evaluation of fetal lung samples from the prenatal developmental toxicity study in rats did not reveal any morphological abnormalities that could have contributed to the sulfoxaflor-induced neonatal mortality in rat pups.

In mechanistic studies conducted to test the hypothesis that the limb abnormalities and bent clavicles in rat fetuses are mediated by the pharmacological agonist action of sulfoxaflor at the fetal neuromuscular junction nAChR, radioligand binding and electrophysiological examination revealed that sulfoxaflor is an agonist of the rat fetal muscle nAChR (which contains the rat γ subunit), whereas it has no agonist activity on the equivalent human fetal nAChR (containing the human γ subunit) or on the rat or human adult muscle nAChR (containing the rat or human ε subunit). In rodents, replacement of the γ subunit by the ε subunit commences late during the 1st postnatal week and is largely complete by the end of the 2nd postnatal week, whereas in humans, the switch from γ to ε subunit expression occurs predominantly during the late fetal period. These results were considered

to support the hypothesis that sulfoxaflor induces fetal abnormalities and neonatal death in rats via its pharmacological action on the fetal muscle nAChR. This receptor develops functional expression between GDs 16 and 17 in the rat, resulting in synchronized fetal limb movements and diaphragmatic responsiveness important for the transition to extrauterine respiration.

Two developmental toxicity range-finding studies in rabbits demonstrated that administration of sulfoxaflor in the diet afforded a greater applied maximally tolerated dose (1000 ppm, equal to 36.6 mg/kg bw per day) relative to gavage (15 mg/kg bw per day caused excessive maternal toxicity). Thus, dietary administration of sulfoxaflor was chosen for the main developmental toxicity study.

In a developmental toxicity study in rabbits, the NOAEL for maternal toxicity was 150 ppm (equal to 6.6 mg/kg bw per day), based on decreased faeces and decreases in body weight gain and feed consumption at 750 ppm (equal to 31.9 mg/kg bw per day). The NOAEL for prenatal developmental toxicity was 750 ppm (equal to 31.9 mg/kg bw per day), the highest dose tested.

In a special study conducted to assess the effects of sulfoxaflor on neonatal survival in rabbits, dams were exposed to sulfoxaflor (750 ppm, equal to 29 mg/kg bw per day) from gestation day 7 through the initiation of parturition and allowed to deliver and rear their offspring to lactation day 4. Dams showed decreased body weight gains and feed consumption, whereas no treatment-related effects on the mean number of offspring born, offspring survival or the general physical condition of the offspring were observed.

The Meeting concluded that for the limb abnormalities and bent clavicles observed in rats, there is sufficient evidence that these effects were induced by pharmacological action of sulfoxaflor at the rat fetal muscle nAChR, whereas sulfoxaflor has no agonist activity on the equivalent human fetal nAChR or on the rat or human adult muscle nAChR. This allowed for the conclusion that these effects are not relevant to humans. Regarding the reduced neonatal survival observed in rats, the Meeting noted that the human relevance for this effect cannot be excluded, as the underlying MOA is unclear.

In an acute neurotoxicity study in rats, the NOAEL for neurotoxicity was 25 mg/kg bw, based on decreased motor activity at 75 mg/kg bw. There was no evidence for neuropathological effects up to the highest dose tested (750 mg/kg bw).

In a developmental neurotoxicity study in rats, the NOAEL for maternal and reproductive toxicity was 400 ppm (equal to 28.8 mg/kg bw per day), the highest dose tested. The NOAEL for developmental neurotoxicity was 400 ppm (equal to 28.8 mg/kg bw per day), as there were no signs of developmental neurotoxicity at any exposure level. The NOAEL for neonatal toxicity was 100 ppm (equal to 7.4 mg/kg bw per day), based on the reduction in postnatal survival and pup body weights at 400 ppm (equal to 28.8 mg/kg bw per day).

Toxicological data on metabolites

X11719474, the major soil and plant metabolite of sulfoxaflor, was of low acute oral toxicity in rats (LD_{50} > 5000 mg/kg bw) and showed no genotoxic potential in vitro in mammalian or microbial test systems. In a 90-day oral toxicity study in rats, the NOAEL was 1000 ppm (equal to 65.3 mg/kg bw per day), based on effects in the liver (vacuolation/fatty change) at 5000 ppm (equal to 327 mg/kg bw per day). In a reproduction toxicity screening study in rats, the NOAEL for reproductive and offspring performance was 5000 ppm (equal to 396 mg/kg bw per day), the highest dose tested. In a prenatal developmental toxicity study in rats, the NOAEL for developmental toxicity was 5000 ppm (equal to 368 mg/kg bw per day), the highest dose tested.

X11721061, a plant and animal (rat) metabolite of sulfoxaflor, was of low acute oral toxicity in rats (LD_{50} = 2000 mg/kg bw) and showed no genotoxic potential in vitro in mammalian or microbial test systems. In a 28-day oral toxicity study in rats, the NOAEL was 3000 ppm (equal to 236 mg/kg bw per day), based on reduced feed consumption at 8000 ppm (equal to 622 mg/kg bw per day).

X11596066, a metabolite of sulfoxaflor identified in hens and goats, was of low acute oral toxicity in rats (LD_{50} > 2000 mg/kg bw) and showed no genotoxic potential in vitro (Ames test).

X11579457, a soil metabolite of sulfoxaflor, was of low acute oral toxicity in rats (LD_{50} > 2000 mg/kg bw) and showed no genotoxic potential in vitro in mammalian or microbial test systems.

X11519540, a soil and animal (hen) metabolite of sulfoxaflor, was of moderate acute oral toxicity in rats (LD_{50} = 565 mg/kg bw) and showed no genotoxic potential in vitro in mammalian or microbial test systems. In a 28-day oral toxicity study in rats, the NOAEL was less than 100 ppm (equal to 7.7 mg/kg bw per day) in males, based on effects in liver (increased serum cholesterol), thyroid (follicular cell hypertrophy) and adrenals (increased vacuolation of the cortex).

All metabolites were less toxic than the parent compound, except for X11519540, which had higher acute and higher short-term toxicity than the parent.

There were no reports of adverse health effects in manufacturing plant personnel. Also, there were no reports of poisonings with sulfoxaflor.

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

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) for sulfoxaflor of 0–0.05 mg/kg bw, based on a NOAEL of 5.13 mg/kg bw per day for hepatocellular degeneration in female rats in a 2-year toxicity and carcinogenicity study and application of a safety factor of 100. The ADI is supported by the NOAEL of 6.07 mg/kg bw per day for systemic toxicity (increased vacuolation/fatty change of centrilobular hepatocytes in F_0 males) and offspring toxicity (reduced neonatal survival) at 24.6 mg/kg bw per day in a two-generation rat study, the NOAEL of 6.36 mg/kg bw per day, based on effects in the liver (increased serum cholesterol, vacuolation/fatty change of hepatocytes) in a 13-week study in rats, and the overall NOAEL of 6 mg/kg bw per day in the 90-day and 1-year dog studies.

The Meeting established an acute reference dose (ARfD) for sulfoxaflor of 0.3 mg/kg bw, based on the NOAEL of 25 mg/kg bw for decreased motor activity at 75 mg/kg bw in an acute neurotoxicity study in rats. A 100-fold safety factor was applied.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Thirteen-week study of toxicity	Toxicity	100 ppm, equal to 12.8 mg/kg bw per day	750 ppm, equal to 98 mg/kg bw per day
	Eighteen-month study of toxicity and carcinogenicity	Toxicity	100 ppm, equal to 10.4 mg/kg bw per day	750 ppm, equal to 79.6 mg/kg bw per day
		Carcinogenicity	100 ppm, equal to 10.4 mg/kg bw per day	750 ppm, equal to 79.6 mg/kg bw per day
Rat	Thirteen-week study of toxicity	Toxicity	100 ppm, equal to 6.36 mg/kg bw per day	750 ppm, equal to 47.6 mg/kg bw per day
	Two-year study of toxicity and carcinogenicity	Toxicity	100 ppm, equal to 5.13 mg/kg bw per day	750 ppm, equal to 39.0 mg/kg bw per day
		Carcinogenicity	100 ppm, equal to 4.24 mg/kg bw per day[a]	500 ppm, equal to 21.3 mg/kg bw per day

Species	Study	Effect	NOAEL	LOAEL
	Two-generation study of reproductive toxicity	Reproductive toxicity	400 ppm, equal to 24.6 mg/kg bw per day[b]	—
		Parental toxicity	100 ppm, equal to 6.07 mg/kg bw per day	400 ppm, equal to 24.6 mg/kg bw per day
		Offspring toxicity	100 ppm, equal to 6.07 mg/kg bw per day	400 ppm, equal to 24.6 mg/kg bw per day
	Developmental toxicity study	Maternal toxicity	150 ppm, equal to 11.5 mg/kg bw per day	1000 ppm, equal to 70.2 mg/kg bw per day
		Embryo and fetal toxicity	150 ppm, equal to 11.5 mg/kg bw per day	1000 ppm, equal to 70.2 mg/kg bw per day
	Acute neurotoxicity study[c]	Neurotoxicity	25 mg/kg bw	75 mg/kg bw
	Developmental neurotoxicity study	Developmental neurotoxicity	400 ppm, equal to 28.8 mg/kg bw per day[b]	—
Rabbit	Developmental toxicity study	Maternal toxicity	150 ppm, equal to 6.6 mg/kg bw per day	750 ppm, equal to 31.9 mg/kg bw per day
		Embryo and fetal toxicity	750 ppm, equal to 31.9 mg/kg bw per day[b]	—
Dog	Thirteen-week and 1-year studies of toxicity[c,d]	Toxicity	6 mg/kg bw per day	10 mg/kg bw per day

[a] Not considered relevant for human risk assessment.
[b] Highest dose tested.
[c] Gavage administration.
[d] Two studies combined.

Estimate of acceptable daily intake for humans

0–0.05 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 sulfoxaflor

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid; ≥ 93%
Distribution	Extensive; highest concentrations in liver, kidney and erythrocytes
Rate and extent of excretion	≥ 93% within 168 h (≥ 92% in urine; 5–9% in faeces)
Potential for accumulation	None
Metabolism in animals	Very limited, oxidative cleavage of the molecule, followed by conjugation with glucuronic acid; one metabolite identified in urine (conjugate of X11721061, 3–4% of administered dose)
Toxicologically significant compounds (animals, plants and the environment)	Sulfoxaflor, X11519540 (soil metabolite)

Acute toxicity	
Rat, LD_{50}, oral	1000 mg/kg bw
Rat, LD_{50}, dermal	> 5000 mg/kg bw
Rat, LC_{50}, inhalation	> 2.09 mg/l (4 h, nose-only exposure)
Rabbit, dermal irritation	Not irritating
Rabbit, eye irritation	Not irritating
Mouse, dermal sensitization (local lymph node assay)	Not sensitizing
Short-term studies of toxicity	
Target/critical effect	Liver (liver cell vacuolation/fatty change) in mice and rats; adrenals (cortical hypertrophy and vacuolation) in mice; decreased feed consumption and body weight gain in dogs
Lowest relevant oral NOAEL	6.36 mg/kg bw per day (90-day study in rats)
Lowest relevant dermal NOAEL	500 mg/kg bw per day (28-day study in rats)
Lowest relevant inhalation NOAEC	No data
Long-term toxicity and carcinogenicity	
Target/critical effect	Liver (liver cell vacuolation/fatty change) in mice and in female rats
Lowest relevant NOAEL	5.13 mg/kg bw per day (2-year study in rats)
Carcinogenicity	Unlikely to pose a carcinogenic risk to humans at levels of dietary exposure
Genotoxicity	
	Not genotoxic
Reproductive toxicity	
Reproductive target/critical effect	No effects on fertility at highest dose tested; reduced pup survival and delayed preputial separation at parentally toxic dose
Lowest relevant reproductive NOAEL	6.07 mg/kg bw per day for offspring toxicity (two-generation study in rats)
Developmental target/critical effect	Fetal abnormalities (forelimb flexure, hindlimb rotation, bent clavicle, fused sternebrae, convoluted ureter) at maternally toxic dose
Lowest relevant developmental NOAEL	11.5 mg/kg bw per day (rat)
Neurotoxicity	
Acute neurotoxicity	Decrease in motor activity; NOAEL: 25 mg/kg bw
Developmental neurotoxicity	No evidence of developmental neurotoxicity at highest dose tested
Other toxicological studies	
Mechanistic studies	Studies on liver tumorigenesis (rats, mice) demonstrate non-relevance to humans Studies on Leydig cell effects in rats suggested evidence for an MOA unlikely to be relevant to humans Studies demonstrated that limb and clavicle abnormalities are due to rat-specific agonist activity on the fetal muscle nAChR not relevant to humans

Studies on metabolites	X11719474: lower toxicity than parent compound, not genotoxic in vitro, no developmental toxicity X11721061, X11596066 and X11579457: lower toxicity than parent compound, not genotoxic in vitro X11519540: higher toxicity than parent compound, not genotoxic in vitro
Medical data	
	Limited data; no adverse health effects reported in manufacturing plant personnel

Summary

	Value	Study	Safety factor
ADI	0–0.05 mg/kg bw	Two-year study in rat (supported by two-generation study in rats, 90-day study in rats and 1-year study in dogs)	100
ARfD	0.3 mg/kg bw	Acute neurotoxicity study in rat	100

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Appendix 1: Application of the IPCS conceptual framework for cancer risk assessment

(IPCS framework for analysing the relevance of a cancer mode of action for humans)

This framework, developed by an International Programme on Chemical Safety (IPCS) working group, provides a generic approach to the principles commonly used in evaluating a postulated MOA for tumour induction by a chemical (Sonich-Mullin et al., 2001; Boobis et al., 2006). Thus, the framework was used by the 2011 JMPR to provide a structured approach to the assessment of the overall weight of evidence for the postulated MOA for the increased incidences of hepatocellular

adenoma and carcinoma in male mice, the increased incidences of hepatocellular adenoma in male rats and the increased incidences of bilateral Leydig cell adenomas in male rats observed after long-term administration of sulfoxaflor.

A. Hepatocellular tumours in mice and rats

A.1 Introduction

In the 18-month study of carcinogenicity in mice, increased incidences of hepatocellular adenoma and carcinoma were observed in male mice after administration of sulfoxaflor at a dietary concentration of 750 ppm (equal to 79.6 mg/kg bw per day).

In the 24-month study of chronic toxicity and carcinogenicity in rats, increased incidences of hepatocellular adenoma were observed in male rats after administration of sulfoxaflor at a dietary concentration of 500 ppm (equal to 21.3 mg/kg bw per day) (see section 2.3).

A.2 Postulated mode of action (theory of the case)

The postulated MOA for the development of liver cell tumours in rodents after long-term administration of sulfoxaflor is considered to be similar to the MOA for phenobarbital, which induces liver cell tumours in rodents by a non-genotoxic mechanism (LeBaron et al., 2010). A key effect of phenobarbital is the induction of hepatic cytochrome P450 enzymes, which is mediated through activation of nuclear receptors, in particular CAR in hepatocytes (and possibly, to some extent, PXR), which is followed by altered expression of CAR- (and PXR-)regulated genes and subsequent induction of liver enzymes (including CYP isoenzymes), hepatocellular hypertrophy, increase of cell proliferation and suppression of apoptosis and perturbation of liver function. The increased cell replication and suppression of normal apoptotic processes are associated with clonal expansion of potentially mutated cells, which leads to foci of altered hepatocytes. Such altered foci ultimately progress to neoplasia (Bannasch, Haertel & Su, 2003).

A.3 Key events

Analysis of the available toxicological data for sulfoxaflor, including extensive mechanistic data, suggested a phenobarbital-like induction of hepatic CYP enzymes as the most likely MOA for the liver cell tumour development, which would operate through the following key events:

- *Activation of nuclear receptors, in particular CAR*: Activation of nuclear receptors, in particular CAR, is most probably an early step, although no *direct* evidence has been presented that demonstrates an interaction of sulfoxaflor with any nuclear receptor. However, the activation of CYP2B genes is commonly considered to be a diagnostic, surrogate response to CAR activation. This response was demonstrated in mice treated for 7–90 days and in rats treated for 3–7 days by increased expression of CYP2B10 or CYP2B1 mRNA (in mice or rats, respectively) and by increases in CYP2B10 proteins in mice.
- *Induction of hepatic CYP enzymes*: Induction of hepatic CYP2B enzymes, particularly BROD and PROD, was observed in mice treated for 7–90 days (≥ 44 mg/kg bw per day) and in rats treated for 3–7 days (≥ 59 mg/kg bw per day) and may be considered to be indicative of a CAR-mediated response.
- *Increase of liver cell proliferation*: Increased liver cell proliferation was demonstrated in mice (BrdU labelling, Ki-67 staining; ≥ 89 mg/kg bw per day) and in rats (Ki-67 staining; ≥ 59 mg/kg bw per day) after 7 days of sulfoxaflor administration.
- *Hepatocellular hypertrophy and hyperplasia*: Hepatocellular hypertrophy was observed in mice after 7 or 90 days and 18 months of treatment (128, ≥ 98 or 80 mg/kg bw per day, respectively)

and in rats after 28 or 90 days and 12–24 months of treatment (≥ 79, ≥ 48 or 21 mg/kg bw per day, respectively).

- *Increase in liver weight*: Increased liver weight was observed in mice after 7 or 90 days and 18 months of treatment (128, ≥ 98 or 80 mg/kg bw per day, respectively) and in rats after 7, 28 or 90 days and 12–24 months of treatment (≥ 59, ≥ 79, ≥ 48 or 21 mg/kg bw per day, respectively).
- *Development of altered hepatic foci*: Foci of altered hepatocytes (eosinophilic and vacuolated foci) were increased in male mice after administration of sulfoxaflor for 18 months at 80 mg/kg bw per day. In male rats, the incidence of eosinophilic foci (quantified as more than five foci in the three standard liver sections examined microscopically) was marginally increased at 21 mg/kg bw per day when compared with controls (19 versus 9, respectively).

The key events as described above have been observed in mice and rats in mechanistic studies as well as in short-term and long-term toxicity studies. The dose–response relationships and the temporal analyses of the key events and tumour response are presented below.

A.4 Concordance of dose–response relationships

All of the features considered to contribute to the key events occurred in dose–response relationships, and it was obvious that key events regarded as occurring later in the MOA sequence did not occur at doses lower than those regarded as occurring earlier in the MOA sequence. The NOAELs and lowest-observed-adverse-effect levels (LOAELs) for the key events in the MOA for sulfoxaflor-induced liver tumours in mice and rats are provided in Tables A1 and A2, respectively.

Table A1. NOAELs/LOAELs for key events in the MOA for sulfoxaflor-induced liver cell tumours in mice

Key event	NOAEL/LOAEL (mg/kg bw per day)
Activation of CAR (*indirect* evidence based on activation of CYP2B10 genes)	—/89 (7-day mechanistic study)
	—/44 (28-day study)
	—/98 (90-day study)
Induction of CYP2B enzymes (PROD, BROD)	—/89 (7-day mechanistic study)
	—/44 (28-day study)
	—/98 (90-day study)
Increase in liver cell proliferation	—/418 (7-day palatability study; Ki-67 staining)
	—/89 (7-day mechanistic study; increased BrdU labelling, mitotic alteration)
	44/230 (28-day study; mitotic alteration)
	98/166 (90-day study; mitotic alteration)
	10/80 (18-month study; mitotic alteration)
Hepatocellular hypertrophy	—/89 (7-day mechanistic study)
	44/230 (28-day study)
	13/98 (90-day study)
	10/80 (18-month study)
Increase in liver weight	89/128 (7-day mechanistic study)
	44/230 (28-day study)
	13/98 (90-day study)
	2.5/10 (18-month study)
Increase in hepatic foci	10/80 (18-month study; increase in eosinophilic and vacuolated)
Increase in liver cell tumours	10/80 (18-month study; increase in adenoma and carcinoma)

Table A2. NOAELs/LOAELs for key events in the MOA for sulfoxaflor-induced liver cell tumours in rats

Key event	NOAEL/LOAEL (mg/kg bw per day)
Activation of CAR (*indirect* evidence based on activation of CYP2B1 genes)	—/8.9 (3-day mechanistic study) —/8.0 (7-day mechanistic study)
Induction of CYP2B enzymes (PROD, BROD)	8.9/60 (3-day mechanistic study) 8.0/59 (7-day mechanistic study)
Increase in liver cell proliferation	99/— (3-day mechanistic study; Ki-67 staining) 8.0/59 (7-day mechanistic study; Ki-67 staining) —/192 (28-day study; Ki-67 staining)
Hepatocellular hypertrophy	25/79 (28-day study) 6.4/48 (90-day study) 4.2/21 (24-month study)
Increase in liver weight	60/99 (3-day mechanistic study) 8.0/59 (7-day mechanistic study) 25/79 (28-day study) 6.4/48 (90-day study) 4.2/21 (24-month study)
Increase in hepatic foci	4.2/21 (24-month study; marginal increase in eosinophilic foci)
Increase in liver cell tumours	4.2/21 (24-month study; increase in adenoma)

In mice, activation of CYP2B10 genes as well as increases of hepatic CYP2B enzyme activities (PROD, BROD) and of liver cell proliferation were observed in a dose-related manner in a 7-day mechanistic study (at ≥ 89 mg/kg bw per day). Also, the increases in the incidence and/or severity of hepatocellular hypertrophy and of liver weights were dose related in the 28- and 90-day toxicity studies and in the 18-month carcinogenicity study.

In rats, dose-related activation of CYP2B1 genes as well as increases of hepatic CYP2B enzyme activities (PROD, BROD), of liver cell proliferation and of liver weights were demonstrated in both a 3-day and 7-day mechanistic study (activation of CYP2B1 genes at ≥ 8 mg/kg bw per day; other effects at ≥ 59 mg/kg bw per day). Also, the increases in the incidence or severity of hepatocellular hypertrophy and of liver weights were dose related in the 28- and 90-day toxicity studies (at 48–79 mg/kg bw per day and above), whereas these effects were noted in the 24-month toxicity and carcinogenicity study only at the highest dose tested (21 mg/kg bw per day).

A.5 Temporal association

Within the limitations of study design, the temporal relationships followed a logical pattern. The key events occurring earlier in the MOA sequence, such as activation of CAR, induction of CYP2B enzymes, increased liver cell proliferation, liver cell hypertrophy and increased liver weights, were observed after only a 7-day or 28-day exposure to sulfoxaflor. In mice, increased incidences of foci of altered hepatocytes (and adenoma/carcinoma) first appeared after 18 months of exposure, whereas in rats, a marginal increase of eosinophilic foci and increased incidences of liver cell adenoma were observed after 24 months of exposure.

A.6 Strength, consistency and specificity of association of tumour response with key events

Strength, consistency and specificity of the association can be established from the studies described previously. The quantifiable precursor events, fundamental to the proposed MOA, are

relatively consistent with the emergence of liver cell tumours. Induction of CYP enzyme activity is a well-known MOA for rodent hepatocarcinogens, and phenobarbital is a standard example of a CAR-mediated CYP inducer (Whysner, Ross & Williams, 1996; Holsapple et al., 2006; Lake, 2009; Cohen, 2010). The key events for this MOA are CAR activation, with associated CYP isozyme induction, and increase in hepatocellular proliferation, which result in subsequent adenomas and carcinomas. In addition to these key events, reversibility of hepatic effects upon discontinuance of treatment is considered necessary information to support this MOA (Cohen, 2010).

Overall, the mechanistic studies for both mice and rats clearly demonstrate a sulfoxaflor-induced, dose-related increase in the *Cyp2b*/CAR-associated transcript and associated increase in specific CYP2B protein (CYP2B10 in mice and CYP2B1 in rats) and enzymatic activity (PROD/BROD). These results are consistent with the direct activation of the CAR nuclear receptor. In addition, analysis of hepatocellular proliferation indicates a clear, dose-related induction of S-phase DNA synthesis. Both of these key events were demonstrated to be directly tied to the activity of the CAR nuclear receptor by the use of genetically modified mouse models (i.e. CAR/PXR-null, knockout, CARKO/PXRKO), where no CAR activity (gene or protein expression of CYP2B10) or increase in hepatocellular proliferation was noted at a carcinogenic dose level of 750 ppm (equal to 99–120 mg/kg bw per day). Furthermore, the gross and microscopic hypertrophic effects of sulfoxaflor on the liver were reversible upon removal of the test material. Lastly, the CYP2B/CAR-associated gene expression and protein data from these MOA experiments in both mice and rats define a plausible sulfoxaflor MOA, while simultaneously ruling out other nuclear receptor–mediated MOAs for rodent hepatic carcinogens, such as PPARα or AhR agonism.

A.7 Biological plausibility and coherence

The proposed MOA for the induction of liver tumours in male mice and rats by sulfoxaflor is plausible and cohesive, as the data show a substantial similarity to the MOA that has been proposed (and which is widely accepted) for phenobarbital.

In addition, the specificity for the MOA was demonstrated for sulfoxaflor using genetically engineered mouse models. As described above, the CARKO/PXRKO mice were refractory to the CAR-mediated hepatic effects demonstrated for sulfoxaflor in wild-type mice. Moreover, and most importantly, "humanized" CAR/PXR (hCAR/hPXR) mice demonstrated a similar, although quantitatively lower, response for most end-points directly associated with CAR activation, but no increase in hepatocellular proliferation was noted. These data are consistent with the known MOA for phenobarbital and other CAR activators and are considered to be supportive of why humans are refractory to the hepatocarcinogenic effects of phenobarbital (Holsapple et al., 2006; Lake, 2009; Cohen, 2010).

A.8 Other modes of action

a) Genetic activity, including DNA reactivity, is always one possible MOA to consider, but no genotoxic potential was demonstrated for sulfoxaflor in the following tests:

   - bacterial reverse mutation test,
   - mammalian cell gene mutation test,
   - mammalian chromosomal aberration test, in vitro,
   - mammalian erythrocyte micronucleus test, in vivo.

   Therefore, the available evidence indicates that genotoxicity is not an alternative MOA for sulfoxaflor.

b) There was no indication of hepatotoxic effects such as peroxisome proliferation or chronic degeneration in the general toxicity studies performed on sulfoxaflor that might suggest cycles of degeneration and regenerative hyperplasia.

c) Also, none of the studies of general toxicity or toxicity to reproduction have suggested that there might be perturbation of estrogenic hormone homeostasis that could result in a mitogenic stimulus to the liver.

d) An unknown is whether CYP induction is a surrogate for a wider pleiotrophic response (Ueda et al., 2002); it is known that CAR is involved in the epigenetic alteration of a large number of different genes, many of which may be involved in tumorigenesis (Phillips & Goodman, 2009; Phillips, Burgoon & Goodman, 2009).

A.9 Uncertainties, inconsistencies and data gaps

No direct evidence for binding to CAR or rather activation of CAR by sulfoxaflor has been provided. However, this data gap might be considered to be of low relevance given the close similarities in the MOA between sulfoxaflor and phenobarbital, which is considered to be the prototype for rodent liver tumour formation by CAR-mediated induction of hepatic CYP2B forms.

Also, the MOA evaluation focused mainly on observations in male mice and rats (which were more sensitive than female mice and rats to hepatic effects of sulfoxaflor), although a statistically not significantly increased incidence of hepatocellular tumours was identified in female mice treated for 18 months at 1250 ppm (equal to 176 mg/kg bw per day). Histopathological examination of the liver of those animals with hepatocellular tumours (and of liver tissue in the shorter-duration studies) revealed a phenotype entirely consistent with that identified in males of increased CYP induction and eosinophilia. Although inclusion of females in the MOA studies and MOA evaluation may have been informative, the MOA data provide compelling evidence that the sulfoxaflor liver tumour MOA is not sex specific. Thus, restricting the MOA investigations to the more sensitive sex significantly reduced the number of animals used for the studies.

Reversibility of sulfoxaflor-induced hepatic effects was investigated in a standard, repeated-dose 90-day rat toxicity study. Animals administered the top dietary concentration of 1500 ppm (equal to 95 mg/kg bw per day, i.e. more than 4-fold greater than the hepatocarcinogenic dose level of 21 mg/kg bw per day in the 2-year rat study) for 90 days had a relative liver weight increase of 41% with clear microscopic hepatocellular hypertrophy identified. A subset of these animals was then subsequently switched to a control diet for an additional 28 days, and the data indicated that those animals did not have significantly increased relative liver weights or microscopic hepatocellular hypertrophy compared with controls. A complete evaluation of the molecular reversibility for sulfoxaflor-induced hepatic effects across all MOA studies was not undertaken in an effort to restrict animal usage, as the most definitive experiment for specificity of sulfoxaflor-induced liver effects was demonstrated with the use of CARKO/PXRKO (knockout) and hCAR/hPXR (humanized) mice.

A.10 Assessment of postulated mode of action

The data provided support a non-genotoxic, threshold-based MOA for the development of liver cell tumours in mice and rats following chronic exposure to sulfoxaflor. The key events for the proposed MOA of sulfoxaflor have been identified and documented, and they illustrate a strong dose–response relationship and temporal relationship to tumour formation. The proposed MOA of sulfoxaflor is consistent with the well-known MOA for phenobarbital-induced liver tumours in rodents and is consistent with the current understanding of cancer biology and nuclear receptor–mediated carcinogenesis.

Human relevance analysis

1. Is the weight of evidence sufficient to establish a mode of action in animals?

The toxicological data provided for sulfoxaflor support a non-genotoxic, threshold-based MOA for the development of liver cell tumours in mice and rats, and the weight of evidence supports a phenobarbital-like MOA.

2. Can human relevance of the MOA be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans?

The key events in CAR-mediated hepatocellular carcinogenesis include activation of CAR and induction of CYP isozymes, leading to increased hepatocellular proliferation with subsequent induction of proliferative lesions in the liver, including foci, adenomas and carcinomas. Activation of rodent CAR produces a cascade of alterations in gene transcription that leads to increased hepatocellular proliferation, a critical event in the development of liver tumours (Whysner, Ross & Williams, 1996; Cohen, 2010).

In contrast, phenobarbital-induced enzyme induction in human liver may act more through PXR than through CAR (Moore et al., 2003), and different enzymes are induced in humans compared with rodents (Lambert et al., 2009). Also, and more importantly, there is no evidence of increased hepatocellular proliferation in humans or primary human hepatocytes in vitro after exposure to phenobarbital (Lake, 2009). Furthermore, recent research has strongly suggested that human CAR and PXR introduced to mouse knockout models lacking the mouse genes for these functions result only in hepatic hypertrophy and not hepatic hyperplasia when these modified mice are treated with either phenobarbital or chlordane (Ross et al., 2010). This finding was confirmed in studies with sulfoxaflor, where humanized CAR/PXR knock-in mice were refractory to the hepatocellular proliferative effect of sulfoxaflor, whereas wild-type mice demonstrated increased proliferation.

Extensive epidemiological studies in humans exposed to levels of phenobarbital comparable to those in rodent bioassays did not find an increased risk of cancer (Whysner, Ross & Williams, 1996; Lamminpaa et al., 2002). Based on the MOA assessment, phenobarbital is not a hepatocarcinogen in humans. Furthermore, a hepatocarcinogenic response in rodents for compounds that have data to support a phenobarbital-like MOA is not relevant to humans (Holsapple et al., 2006). On this basis, the rodent liver tumours associated with administration of high dose levels of sulfoxaflor would not pose a cancer hazard to humans.

3. Can human relevance of the MOA be reasonably excluded on the basis of fundamental, quantitative differences in key events between experimental animals and humans?

As human relevance of the experimental animal MOA can be reasonably excluded on the basis of qualitative differences in key events (Question 2), a quantitative assessment of kinetic or dynamic factors is not necessary.

B. Leydig cell adenomas in rats

B.1 Introduction

In the 24-month study of chronic toxicity and carcinogenicity in Fischer 344 rats, increased incidences of Leydig cell adenoma were observed in male rats after administration of sulfoxaflor at a dietary concentration of 500 ppm (equal to 21.3 mg/kg bw per day) (see section 2.3).

B.2 Postulated mode of action (theory of the case)

The postulated MOA for the increase of Leydig cell tumours in rats after long-term administration of sulfoxaflor is based on the hypothesis that sulfoxaflor can act as a dopamine agonist in the brain and reduces prolactin release by the anterior pituitary gland (Rasoulpour et al., 2011). This results in decreased serum prolactin levels and decreased binding of prolactin to prolactin receptors on Leydig cells, which leads to a downregulation of LHR on Leydig cells. The LHR downregulation results in decreased testosterone production, which feeds back to induce LH release from the pituitary and causes a compensatory increase in circulating LH. The elevation in LH produces Leydig cell hyperplasia and Leydig cell tumours if the elevations are over a sustained period.

B.3 Key events

Following analysis of the available toxicological data for sulfoxaflor, including extensive mechanistic data, dopamine agonism was proposed as the most likely MOA for the Leydig cell tumour development in Fischer 344 rats, which would operate through the following key events:

1) increased neuronal dopamine release via specific dopaminergic neuron-based nAChR agonism,
2) decreased serum prolactin levels,
3) downregulation of LHR gene expression in Leydig cells,
4) transient decreases in serum testosterone,
5) increased serum LH levels,
6) increase in Leydig cell proliferation.

This hypothesis was evaluated in a specific MOA study in which these key events were examined to determine the causality of sulfoxaflor's promotion of Fischer 344 rat Leydig cell tumours in the carcinogenicity study. Additionally, studies were conducted to consider whether other known potential MOAs were involved in the Leydig cell tumour promotion effect of sulfoxaflor and to examine the effect of sulfoxaflor on extracellular levels of dopamine in the mediobasal hypothalamus.

B.4 Concordance of dose–response relationships

With respect to dose–response relationships, owing to the subtle nature of the effects, no precursor key events were observed at 100 ppm (equal to 4.2–5.6 mg/kg bw per day), only at 500 ppm (equal to 21–28 mg/kg bw per day). A dose–response relationship for these apical end-point effects existed with increased testis size and increased incidence of bilateral Leydig cell tumours at 500 ppm. Because of the high background incidence of these tumours in Fischer 344 rats, the lack of a response for precursor key events with the MOA analysis at the 100 ppm dose level is not surprising.

The LOAELs for the key effects in the MOA of sulfoxaflor in the Leydig cells are provided in Table B1.

Key event #1 (increased dopamine release) has been confirmed in a study using intracerebral microdialysis in rats. In this study, sulfoxaflor infusion (0.4 and 2 mmol/l) evoked dose-related increases in the extracellular level of dopamine in the mediobasal hypothalamus, with a maximal rise of 39% 40 minutes after the onset of infusion at 2 mmol/l.

Key event #2 (decreased serum prolactin levels) was demonstrated in the 8-week mechanistic study at the highest dose tested, 28 mg/kg bw per day.

Table B1. NOAELs/LOAELs for key events in the MOA for sulfoxaflor-induced Leydig cell tumours in rats

Key event	NOAEL/LOAEL (mg/kg bw per day)
Increased dopamine release	—/0.4 mmol/l by intracerebral microdialysis
Decreased serum prolactin levels	5.6/28 (8-week mechanistic study: effect observed at week 4, not at week 2 or 8)
Downregulation of LHR gene expression in Leydig cells	5.6/28 (8-week mechanistic study: effect observed at week 4, not at week 8)
Transient decrease in serum testosterone levels	Not demonstrated (8-week mechanistic study) 6.1/25 (two-generation study: delayed preputial separation, probably due to reduced testosterone levels)
Increased serum LH levels	5.6/28 (8-week mechanistic study: effect observed at week 4, not at week 2 or 8)
Increase in Leydig cell proliferation	1.0/4.2 (24-month study: indirect evidence from increased testes weights)
Increase in Leydig cell tumours	4.2/21 (24-month study)

The subsequent key event #3 (downregulation of LHR gene expression in Leydig cells), key event #4 (transient decrease in serum testosterone levels) and key event #5 (increased serum LH levels) were observed also in the same dose range of 25–28 mg/kg bw per day. Key event #4 (transient decrease in serum testosterone levels), however, was not demonstrated directly, but was deduced from indirect evidence (delayed pubertal onset in male rats).

The ultimate key event #6 (Leydig cell proliferation) was also not demonstrated directly; however, a dose–response relationship for this apical end-point effect existed in the 24-month toxicity and carcinogenicity study with increased testes weights at dose levels of 4.2 mg/kg bw per day and above and increased incidence of bilateral Leydig cell adenoma at 21 mg/kg bw per day.

5. Temporal association

Temporal relationships for each of the key events are difficult to ascertain when evaluating hormone data or hormone-dependent changes in target organs, due to the inherent variability, feedback compensation and very long latency for the apical end-point effect of Leydig cell hyperplasia and tumours. Owing to the persistent compensatory nature of the hypothalamic–pituitary–gonadal (HPG) axis, it is not surprising that the changes observed in the hormone data were temporal in nature, whereas conclusive Leydig cell effects occurred only after 2 years of exposure to sulfoxaflor.

6. Strength, consistency and specificity of association of tumour response with key events

The biological processes resulting in rat Leydig cell tumours have been reviewed extensively (Prentice & Miekle, 1995; Clegg et al., 1997; Cook et al., 1999). Leydig cell tumours initially appear as hyperplasia of interstitial cells that can grow with age to the diameter of a single normal seminiferous tubule, at which point they are classified as adenomas per guidance from the United States National Toxicology Program (NTP) (Boorman, Hamlin & Eustis, 1987; Boorman, Chapin & Mitsumori, 1990).

In the 8-week mechanistic study in Fischer 344 rats, there was a dose-dependent increase in LH concentrations concomitant with a dose-dependent decrease in prolactin levels at the 4-week time point, whereas no effects of treatment on prolactin, LH or testosterone levels were observed at all other time points. Consistent with the decreased prolactin levels was a dose-dependent decrease in LHR gene expression at the 4-week, but not 8-week, time point. Although not statistically significant,

the magnitude of gene expression changes is consistent with the dynamic range of these genes in vivo and likely represents a biologically meaningful effect based on alterations in hormone levels.

The specificity of the data for the proposed MOA is the decrease in circulating serum prolactin levels and decreased LHR gene expression. These findings would be observed only with the proposed MOA, but would not be associated with the other possible MOAs leading to Leydig cell tumours (see 8: Other modes of action). Furthermore, the decrease in serum prolactin was associated with a compensatory increase in serum LH, which in turn could act as the primary trophic stimulus over a 2-year Fischer 344 rat carcinogenicity study leading to Leydig cell tumour promotion.

7. Biological plausibility and coherence

Dietary administration of sulfoxaflor to Fischer 344 rats resulted in the early key events (decrease in serum prolactin and LHR gene expression) that lead to an increase in serum LH levels. The MOA demonstrated for sulfoxaflor is for the most part consistent with well-known MOAs for dopamine agonists/enhancers and is consistent with current understanding of hormone-based Leydig cell tumorigenesis. The data for sulfoxaflor are consistent with a non-genotoxic, threshold MOA.

8. Other modes of action

a) Genetic activity, including DNA reactivity, is always one possible MOA to consider, but no genotoxic potential was demonstrated for sulfoxaflor in the following tests:

- bacterial reverse mutation test,
- mammalian cell gene mutation test,
- mammalian chromosomal aberration test, in vitro,
- mammalian erythrocyte micronucleus test, in vivo.

Therefore, the available evidence indicates that genotoxicity is not an alternative MOA for sulfoxaflor.

b) AR antagonists compete with testosterone and DHT for binding to the AR. This competition reduces the androgenic signal to the hypothalamus and adenohypophysis, resulting in an increase in LH secretion with a concomitant elevation of testosterone secretion, leading to the development of Leydig cell tumours (Cook et al., 1999). Direct data obtained with sulfoxaflor showed that although there was an indication that sulfoxaflor was a potential binder to a fragment (i.e. ligand binding domain) of the AR in a non-cell-based binding assay, there was no effect on agonism or antagonism within the AR transactivation assay.

c) ER agonists/antagonists result in changes in estradiol levels, which ultimately cause an increase in LH levels, resulting in the development of Leydig cell tumours. Direct data obtained with sulfoxaflor showed no evidence for ER binding and transactivation (agonism and antagonism), even when tested up to very high concentrations in vitro.

d) 5α-Reductase inhibitors result in decreased conversion of testosterone to DHT. This reduces the net androgenic signal received by the hypothalamus and pituitary, thereby causing a compensatory increase in LH levels, resulting in the development of Leydig cell tumours (Cook et al., 1999). The prostate is differentially sensitive to effects on DHT; for example, DHT has 5-fold greater affinity for AR compared with testosterone (Feldman & Feldman, 2001). Because of this, the prostate would be the most sensitive organ to be affected compared with other accessory sex glands. 5α-Reductase inhibitors can reduce prostate size by 20–30%, although testosterone levels can remain normal (Steers, 2001). Based on the weight of evidence, considering both

direct data that show that sulfoxaflor had no effect on testes 5α-reductase gene expression and indirect data generated from the toxicology package that indicated no prostate effect, a 5α-reductase inhibition MOA is not a plausible alternative MOA for the Leydig cell effects seen in Fischer 344 rats after 2 years of treatment with sulfoxaflor.

e) Aromatase inhibition would result in decreased conversion of androstenedione to estrone and testosterone to estradiol. This would result in an increase in LH levels, leading to the development of Leydig cell tumours (Cook et al., 1999). Direct data obtained with sulfoxaflor showed that it was negative for aromatase inhibition when tested up to very high (i.e. super-physiological) concentrations in vitro. In addition, aromatase inhibitors cause effects on mating and fertility indices as well as female reproductive organ weights and histopathology (Cook et al., 1999; Turner et al., 2000). There were no effects on mating, sperm parameters (counts, motility, morphology) or fertility indices in the two-generation reproductive toxicity study.

f) Inhibition of testosterone biosynthesis would result in lower testosterone and estradiol levels and increased LH levels, resulting in the development of Leydig cell tumours (Cook et al., 1999). Direct data were provided for sulfoxaflor from the Leydig cell tumour MOA study, which showed that there was no treatment-related effect on any measured gene in the steroidogenic pathway, including *StAR* (steroidogenic acute regulatory protein), *Cyp11a1* (P450 side-chain cleavage), *Cyp17a1* (17α-hydroxylase), *HSD3b* (3β-hydroxysteroid dehydrogenase) and *SDR5a1* (5α-reductase). If reduced testosterone biosynthesis were the operant MOA, one or more of these genes would be affected. Also, the hormone panel data would have shown a sustained decrease in circulating levels of testosterone, which was not observed in this study. Taken together, these data, as well as a lack of female reproductive effects, refute decreased steroidogenesis as the operant MOA.

g) Increased biliary elimination of testosterone would cause lower testosterone levels and increased LH levels, resulting in the development of Leydig cell tumours. Based on known nuclear receptor–mediated liver effects with sulfoxaflor administration, this MOA was assessed, and direct data were provided from the Leydig cell tumour MOA study. There were no statistically significant or treatment-related differences in the mean [^{14}C]testosterone-derived radioactivity excreted in the bile across all dose groups, per time interval, and bile flow was very similar for the respective dose groups and time intervals. Thus, these data clearly refute biliary elimination of testosterone as the operant MOA.

h) Gonadotropin releasing hormone (GnRH) (luteinizing hormone releasing hormone, or LHRH) agonism would cause both reduced accessory sex gland weights (due to negative feedback HPG axis compensation) as well as histopathological effects in the pituitary gland, as this is the primary site of functional GnRH receptor expression. As mentioned previously, there were no effects on accessory sex gland weights in the sulfoxaflor two-generation reproductive toxicity study, as well as no treatment-related effects on the pituitary gland in any rat toxicity study, including the 2-year rat carcinogenicity study. Based on the weight of evidence, considering indirect data showing no effect on the pituitary gland, a GnRH agonism MOA is not plausible for the Leydig cell effects seen in Fischer 344 rats after 2 years of treatment with sulfoxaflor.

9. Uncertainties, inconsistencies and data gaps

a) Key event #1 (increased dopamine release via agonism on central dopaminergic neuron nAChRs): Owing to the technical and biological complexity of measuring neurotransmitters within the hypothalamic–hypophyseal portal vein system, key event #1 has been confirmed only after intracerebral infusion of sulfoxaflor into the hypothalamus at relatively high concentrations (0.4 and 2 mmol/l). It remains unclear whether and to what extent increases in dopamine release would occur at the dose levels that were associated with increased Leydig cell tumours in the 24-month study of chronic toxicity and carcinogenicity in rats (21.3 mg/kg bw per day).

b) Key event #4 (transient decreases in serum testosterone): Under the conditions of the 8-week mechanistic study in Fischer 344 rats, there were no measurable decreases in serum testosterone. However, the delay in balanopreputial separation in the two-generation reproductive toxicity study may be considered to provide indirect evidence for a transient decrease in testosterone. Although these data are supportive, there are no hormone measurement data that confirm a decrease in serum levels of testosterone.

c) The dose–response relationships for most of the key events have been established only incompletely, as effects were observed only at 500 ppm (equal to 21–28 mg/kg bw per day), but not at 100 ppm (equal to 4.2–5.6 mg/kg bw per day). However, as a result of the high background incidence of Leydig cell tumours in Fischer 344 rats, the lack of precursor key events for this subtle, hormone-based MOA at the lower dietary concentration of 100 ppm is not surprising, given the transient and compensatory nature of hormone regulation in the HPG axis.

d) The temporal relationships for the key events have not been established adequately, as in the 8-week mechanistic study in Fischer 344 rats, effects on serum prolactin levels, LHR gene expression in Leydig cells and serum LH levels were demonstrated only at the 4-week time point, but not at the 2-week and/or 8-week time points. In addition, effects on serum testosterone levels have not been demonstrated at any time point.

10. Assessment of postulated mode of action

The data provided support a non-genotoxic, threshold-based MOA for the development of Leydig cell tumours in rats following chronic exposure to sulfoxaflor. However, the key events for the proposed MOA of sulfoxaflor, the dose–response relationships and the temporal relationships to tumour formation have been identified or demonstrated only in part. For that reason, the weight of evidence is not sufficient to establish the proposed MOA (dopamine agonism) for the Leydig cell tumours induced in rats after chronic exposure to sulfoxaflor.

11. Conclusion

The relevance of the increased Leydig cell tumours in male Fischer 344 rats to humans cannot be discounted, as the results of the mechanistic studies were only partly sufficient to support the proposed hypothesis that sulfoxaflor can act as a dopamine agonist in the central nervous system. In particular, the increased dopamine release at the median eminence of the hypothalamus into the hypothalamic–hypophyseal portal veins to inhibit prolactin release in the anterior pituitary has been demonstrated only after intracerebral infusion of sulfoxaflor into the hypothalamus at relatively high concentrations (0.4 and 2 mmol/l), and there is a lack of a dose–response relationship and/or temporal concordance with key precursor events (e.g. decrease in serum prolactin level, downregulation of LHR on Leydig cells, decrease in serum testosterone and compensatory increase in serum LH level) and tumour incidence. However, the Leydig cell tumours occurred only at high doses, did not occur in mice and would be anticipated to exhibit a threshold.

For the risk assessment of sulfoxaflor, it must therefore be considered that the effect in question is subtle in nature, and the background incidence of Fischer rat Leydig cell tumours is 75–100% in 2-year studies compared with 1–5% in CD rats, even less in CD-1 mice, and orders of magnitude lower (in ranges of less than 0.01%) for humans. These interspecies differences in background incidence are well understood and result from quantitative and qualitative differences of Leydig cell response to hormonal stimuli. Rat Leydig cells contain more than 10-fold more LHR than those of humans, which confers greater sensitivity to slight changes in LH levels. In addition to this quantitative difference, rat, but not human, Leydig cells have both prolactin and GnRH receptors on their surface. Stimulation of rat Leydig cells through both prolactin and GnRH receptors is a rat-specific mechanism

by which Leydig cell tumour formation can occur. For prolactin receptor involvement in Leydig cell tumours, dopamine agonists (e.g. muselergine) reduce prolactin release by the anterior pituitary gland. This results in decreased binding of prolactin to prolactin receptors on Leydig cells, leading to downregulation of the LHR and transient reductions in testosterone production, which feeds back to induce LH release from the pituitary, leading to Leydig cell stimulation and hyperplasia over time.

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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 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 2011 Joint FAO/WHO Meeting on Pesticide Residues (JMPR), which met in Geneva on 20–29 September 2011.

The monographs in this volume summarize the safety data on 12 pesticides that could leave residues in food commodities. These pesticides are acetamiprid, dichlorvos, dicofol, emamectin benzoate, etofenprox, flutriafol, glyphosate, isopyrazam, penthiopyrad, propylene oxide, saflufenacil and sulfoxaflor. 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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